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Doctoral Dissertation

Sirkku Hanski

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Supervisor	Prof. Olli Ikkala
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Abstract

Polyelectrolyte–surfactant complexes are known to form self-assembled structures at nanometer length scale. When polypeptides are used instead of traditional synthetic polymers, another level of structural control is introduced due to the ability of polypeptides to fold into secondary structures. In this thesis, self-assembly and secondary structure formation of selected cationic homopolypeptides and block copolypeptides are studied in ionic complexes with anionic surfactants.

The surfactant architecture as well as the amount of surfactant were varied and their effect on the self-assembly and polypeptide secondary structures was investigated. Articles I, II and III describe the self-assembly of stoichiometric complexes with single and double alkyl tail surfactants. The complexes were found to respond to a simple external trigger, i.e. temperature, by showing structural changes. In addition, when the amount of surfactant was increased from the stoichiometric value, a plasticization effect from solid to soft liquid crystalline material was observed. Plasticization could be important for solid state applications requiring processing.

In Articles IV and V, hierarchical self-assemblies, i.e. materials with structures at different length scales, are described. Two approaches were used. In Article IV, an asymmetric triple-tail lipid was found to induce a helical conformation in the polypeptides and packing of the helices into a layered structure with 2D correlation between the helices, resulting in an oblique lattice. Crystallization of the lipid alkyl tails was found crucial for such structure formation. Heating past the melting temperature of the side-chain crystallites caused a reversible order–order transition from oblique to hexagonal self-assembly. In Article V, a diblock copolypeptide with one cationic block was complexed with surfactants and hierarchical structure-*and*-structure-*within*-structure self-assemblies were observed. The morphology was found to depend on the surfactant architecture, i.e. the amount of branching in the alkyl tail.

In conclusion, this thesis encourages pursuing novel rationally designed self-assemblies based on polypeptides to enable new schemes for biomimetic materials.

Keywords Polypeptide, surfactant, self-assembly

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Tiivistelmä

Polyelektrolyytti-surfaktantti-kompleksien tiedetään muodostavan nanometrimittakaavan itsejärjestyviä rakenteita. Jos perinteisten synteettisten polymeerien sijaan käytetään polypeptidejä, saadaan sekundäärirakenteiden muodostumisen kautta uusia mahdollisuuksia rakenteiden kontrolloimiseen. Tässä väitöskirjassa tutkitaan eräiden kationisten homo- ja lohkopolypeptidien itsejärjestymistä sekä sekundäärirakenteiden muodostumista, kun ne kompleksoidaan ionisidoksella anionisten surfaktanttien kanssa.

Surfaktanttien arkkitehtuuria sekä määrää komplekseissa vaihdeltiin ja tutkittiin niiden vaikutusta itsejärjestymiseen sekä polypeptidien sekundäärirakenteeseen. Artikkeleissa I, II ja III kuvataan stoikiometristen kompleksien itsejärjestymistä, kun surfaktanteissa on yksi tai kaksi alkyylihäntää. Näiden kompleksien huomattiin muuttavan rakennetta yksinkertaisen ulkoisen ärsykkeen, lämpötilan, vaikutuksesta. Surfaktanttien määrää myös kasvatettiin yli stoikiometrisen arvon, mikä sai aikaan plastisoinnin kiinteästä pehmeään nestekiteiseen materiaaliin. Plastisointi voi olla tärkeää, jos materiaalia halutaan käyttää kiinteän tilan sovelluksissa, jotka vaativat materiaalin prosessointia.

Artikkeleissa IV ja V kuvataan hierarkkista itsejärjestymistä eli materiaaleja, joissa on samanaikaisesti usean mittakaavan sisäkkäisiä rakenteita. Töissä käytettiin kahta lähestymistapaa. Artikkelissa IV huomattiin epäsymmetristen kolmihäntäisten lipidien aiheuttavan polypeptideihin helikaalisen konformaation ja heliksien pakkaamisen kerrosrakenteeseen, jossa eri kerroksissa olevien heliksien paikat korreloivat keskenään vinokulmaisessa hilassa. Lipidin alkyylihäntien kiteytymisen huomattiin olevan tärkeää rakenteen muodostumisen kannalta. Materiaalin lämmittäminen yli sivuketjukiteiden sulamislämpötilan aiheutti palautuvan rakennemuutoksen vinokulmaisesta heksagonaaliseen itsejärjestykseen. Artikkelissa V kaksilohkopolypeptidi, jossa toinen lohkoista on kationinen, kompleksoitiin surfaktantin kanssa. Materiaalit muodostivat hierarkkisia rakenteita, jossa ison toistuvan rakenteen sisällä on kaksi pienemmän mittakaavan toistuvaa rakennetta. Rakennemorfologian huomattiin riippuvan kompleksointiin käytetyn surfaktantin arkkitehtuurista eli alkyylihännän haaroittuneisuudesta.

Tämä väitöskirja osoittaa, että polypeptidipohjaiset materiaalit tarjoavat laajat mahdollisuudet itsejärjestymisen säätämiseen ja uusien biomimeettisten materiaalien aikaansaamiseen.

Asiasanat	Polypeptidi, surfaktantti, itsejärjestyminen		
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PREFACE

The research reported in this thesis was carried out in Molecular Materials group, Department of Applied Physics at Aalto University School of Science and Technology, previously known as Helsinki University of Technology. I want to express my deepest gratitude to my instructor and supervisor Prof. Olli Ikkala for the possibility to work in his group and for his enthusiasm, never ending ideas, encouragement and support during the work. I also want to thank Prof. Janne Ruokolainen for advice and guidance especially on the electron microscopy and scattering methods.

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LIST OF PUBLICATIONS

I Ramasubbu Ramani, **Sirkku Hanski**, Ari Laiho, Roman Tuma, Simo Kilpeläinen, Filip Tuomisto, Janne Ruokolainen, Olli Ikkala, *Evidence of PPII-like Helical Conformation and Glass Transition in a Self-Assembled Solid-State Polypeptide−Surfactant Complex: Poly(L-histidine)/Dodecylbenzenesulfonic Acid*, Biomacromolecules **2008**, 9, 1390-1397.

II **Sirkku Hanski**, Susanna Junnila, László Almásy, Janne Ruokolainen, Olli Ikkala, *Structural and Conformational Transformations in Self-Assembled Polypeptide–Surfactant Complexes,* Macromolecules **2008**, 41, 866-872.

III Susanna Junnila, **Sirkku Hanski**, Richard J. Oakley, Sami Nummelin, Janne Ruokolainen, Charl Faul, Olli Ikkala, *Effect of Double-Tailed Surfactant Architecture on the Conformation, Self-Assembly, and Processing in Polypeptide–Surfactant Complexes*, Biomacromolecules **2009**, 10, 2787-2794.

IV **Sirkku Hanski**, Susanna Junnila, Antti J. Soininen, Janne Ruokolainen, Olli Ikkala, *Oblique Structures and Order–Order Transitions in Polypeptide Complexes with PEGylated Triple-Tail Lipids,* Biomacromolecules, **2010**, ASAP (DOI: 10.1021/bm100972m).

V **Sirkku Hanski**, Nikolay Houbenov, Janne Ruokolainen, Dimitra Chondronicola, Hermis Iatrou, Nikos Hadjichristidis, Olli Ikkala, *Hierarchical Ionic Self-Assembly of Rod–Comb Block Copolypeptide–Surfactant Complexes*, Biomacromolecules **2006**, 7, 3379-3384.

In addition to the articles included in this thesis, the author has contributed to the following publications:

1. Hermis Iatrou, Henrich Frielinghaus, **Sirkku Hanski**, Nikos Ferderigos, Janne Ruokolainen, Olli Ikkala, Dieter Richter, Jimmy Mays, Nikos Hadjichristidis, *Architecturally Induced Multiresponsive Vesicles from Well-Defined Polypeptides. Formation of Gene Vehicles***,** Biomacromolecules **2007**, 8, 2173-2181.

2. Susanna Junnila, Nikolay Houbenov, **Sirkku Hanski**, Hermis Iatrou, Nikos Hadjichristidis, Olli Ikkala, *Hierarchical Smectic Self-Assembly of an ABC Miktoarm Star Terpolymer with a Helical Polypeptide Arm,* Macromolecules **2010**, 43, 9071-9076.

AUTHOR'S CONTRIBUTION

Article I

The author participated in designing the experiments as well as in the data analysis. The author had a major contribution in writing the manuscript.

Article II

The author was responsible for the planning and execution of the experiments. The data were analyzed by the author with the exception of the SANS data, which were analyzed by Ph.D. László Almásy. The first version of the manuscript was written by the author.

Article III

The author took part in the planning of the experiments, acted as an instructor for executing the experiments and for the data analysis and participated in the writing of the manuscript.

Article IV

All work including the planning and executing the experimental work and data analysis was made by the author. The first version of the manuscript was also written by the author.

Article V

This work was done in cooperation with Prof. Nikos Hadjichristidis, Ph.D. Hermis Iatrou and M.Sc. Dimitra Chondronicola, who synthesized the polypeptides. The author participated strongly in the planning of the work. The experiments were carried out together with Ph.D. Nikolay Houbenov, except the transmission electron microscopy imaging, which was solely done by the author. The writing of the manuscript was mainly on the author's responsibility.

TABLE OF CONTENTS

ABBREVIATIONS AND SYMBOLS

CD	Circular dichroism
DBSA	Dodecylbenzenesulfonic acid
diC2/6	Bis(2-ethylhexyl) phosphate
diC8	Dioctyl phosphate
diC12	Didodecyl phosphate
diC14-PEG	1,2-Dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-350] (ammonium salt)
diC16-PEG	1,2-Dipalmitoyl-*sn*- glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-350] (ammonium salt)
diC18-PEG	1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-350] (ammonium salt)
diC18*-PEG	1,2-Dioleyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-350] (ammonium salt)
DSA	Dodecylsulfonic acid
DSC	Differential scanning calorimetry
FFT	Fast Fourier transform
FTIR	Fourier transform infrared spectroscopy
PBLG	Poly(γ-benzyl-L-glutamate)
PArg	Poly(L-arginine)
PHis	Poly(L-histidine)
PLys	Poly(L-lysine)
POM	Polarized optical microscopy
PPII	Polyproline type II helix conformation
SAXS	Small-angle x-ray scattering
TEM	Transmission electron microscopy
TFA	Trifluoroacetic acid
WAXS	Wide-angle x-ray scattering

a	Lattice parameter
b	Lattice parameter
d	Interlayer distance
f	Block copolymer volume fraction
N	Degree of polymerization
h	Miller plane index
k	Miller plane index
n	Number of repeat units
m	Number of repeat units
q	Magnitude of the scattering vector ($Å^{-1}$) ($q = \frac{4\pi}{\lambda}\sin\theta$), where θ is half of the scattering angle.
T	Temperature (°C)
v	Volume
w	Weight
x	Number of surfactants vs. polymer repeating unit
y	Number of surfactants vs. polymer repeating unit
z	Number of surfactants vs. polymer repeating unit

φ	Angle between Miller planes
γ	Angle of the oblique lattice
χ	Flory–Huggins parameter

1 Introduction

During the evolution, Nature has developed ways for creating functional materials from relatively few constituent elements. The key is a complex, often hierarchical, structuring of the building units. Over the last decades, these sophisticated and intricate processes have increasingly served as a source of inspiration also for scientists and engineers in designing new materials. Nowadays, when the requirements for materials properties and performance exceed the limits encountered with the traditional approaches of gluing, stitching and fastening parts together, new ideas are sought from the ways of Nature to build materials with extraordinary properties, such as the mechanical properties of silk, the self-cleaning surface in lotus leaves or the strong adhesion of gecko feet. This means that instead of manipulating macroscopic materials to create smaller scale structures like in lithographic techniques (*top-down approach*), the materials are built from small nanoscale construction units, which assemble to produce macroscopic features (*bottom-up approach*). In nature, the build-up of hierarchical structures is based on a variety of forces that drive the self-assembly or self-organization.[#1]

From materials scientists' point of view, molecular self-assembly is a concept which has many definitions, but here it is useful to describe it as a spontaneous organization of structural units on a nanometer scale. The phenomenon is based on competition between attractive and repulsive interactions within a material.[2, 3] Whereas the interactions in natural materials are often complex, block copolymers are a simpler example of materials which exhibit the self-assembling behavior. In block copolymers, there are two or more incompatible blocks, which are connected to each other by a covalent bond.[4] Because of this covalent bonding between the repelling blocks, they cannot escape from each other, but separate on a microscopic level, which leads to nanoscale structures. The structure of the developing phase depends on the degree of polymerization N, the volume fraction of the blocks f, as well as on the Flory–Huggins interaction parameter χ between the blocks.[4-6] In Figure 1, the simplest morphologies for coil–coil diblock copolymers are presented together with a phase diagram. In addition to simple linear diblock copolymers, also multiblock copolymers and polymers with different architectures, like graft, star-shape or dendritic block copolymers form self-assembled structures with more complicated phase behavior.[4-11]

[#]In literature, the terms self-assembly and self-organization do not yet have precise definitions. In this thesis we use the term self-assembly to mean reversible and cooperative assembly of components into ordered nanoscale structures, like in block copolymers, surfactant solutions or liquid crystals due to competing interactions. For block copolymers also the term microphase separation or sometimes nanophase separation can be found in literature. Here, the term self-organization is limited to dissipative systems requiring continuous energy supply for the structure formation.

The size of the structures, achieved by self-assembly in coil-like block copolymers, ranges typically from tens to a couple of hundred nanometers.[5] If smaller structures are desired, i.e., the degree of polymerization N is decreased, the value of the Flory–Huggins parameter needs to be increased in order to generate microphase separation. This can be achieved either by increasing the chemical dissimilarity of the blocks or by replacing one of the coil-like blocks with a rigid polymer.[12-17] In case of the latter, also the phase behavior changes, as there are more forces affecting the self-assembly, like the tendency of the rigid blocks to aggregate or even crystallize.[18]

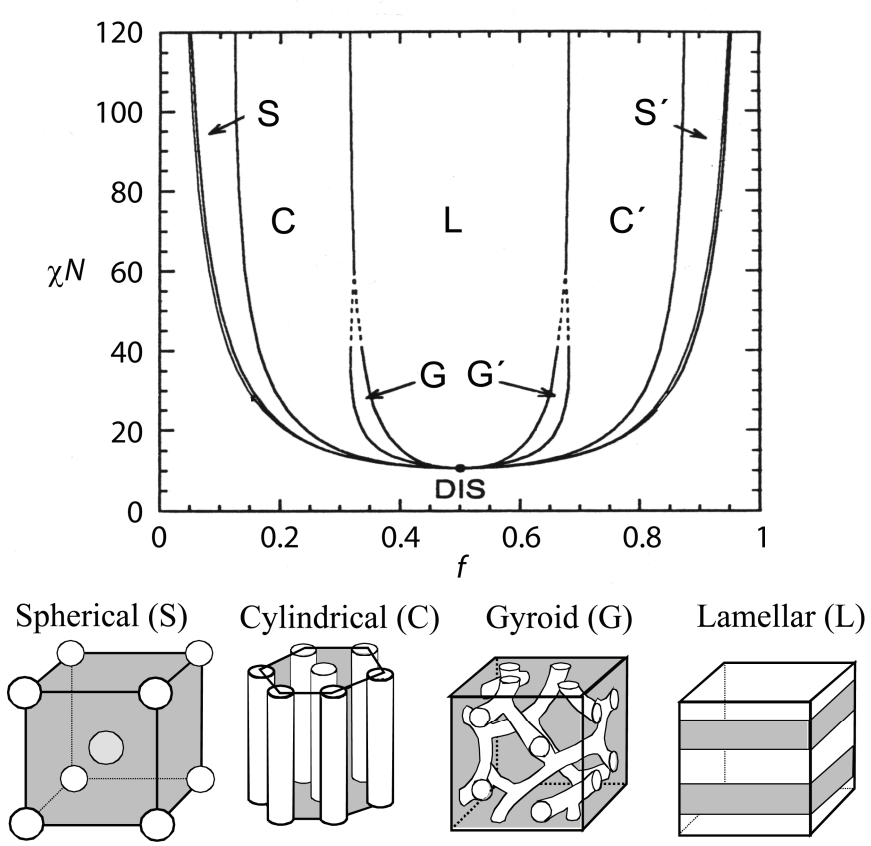

Figure 1. A phase diagram and a schematic illustration of the different morphologies for coil–coil diblock copolymers (redrawn based on ref. 6).

On the other hand, Nature provides construction units that have controlled sizes and shapes. To combine the best from synthetic and biological worlds, scientists have searched for biological motifs to mediate self-assembly towards more complex morphologies and to allow biological functionalities. These include among others helical structures like DNA and RNA,[19-23] well-defined protein assemblies like viruses or virus-like particles[24-26] or protein folding motifs, i.e. secondary and tertiary structures, which are uniform in size and shape.[27] The protein folding motifs can be relatively complicated biologically-inspired peptide sequences, forming naturally occurring structures such as β-sheets, abundant for example in amyloid, silk and elastin, or helical patterns like coiled-coil or collagen structures.[28-32] In addition, already simpler secondary structures, like α-helices or β-sheets based on homopolypeptide sequences, can act as building motifs for self-assembly.[33-39] The topic of this thesis is the use of these small structural units as building blocks for self-assembly and control over the secondary structures as well as the resulting morphologies.

1.1 Outline of the thesis

In this thesis, the ionic self-assembly of different polypeptides and surfactants or lipids is studied. In Chapter 2, we discuss how the different amino acids in the homopolypeptides along with the changes in the surfactant architecture affect the secondary structures of the peptides (see Figure 2 for different building motifs and architectures) and the resulting self-assembled structures (Articles I, II, III). In addition to the stoichiometric ionic complexes, the effect of additional surfactant, interacting with the ionic complex through hydrogen bonds, is studied in order to improve the processability of the intrinsically intractable materials leading to advances also in the self-assembly (Articles II and III). Structural transitions as a response to alterations in temperature are reported in Articles II and III. The transitions are found to be strongly dependent on the surfactant architecture leading to opposite structural behavior in the two articles.

A step forward with the complexity of the systems is taken with Articles IV and V in Chapter 3 where we present two different ways of bringing self-assembled hierarchy, i.e. structural periodicities at different length scales, to the materials. First, the structure of the surfactant is changed from the simple single-tail and double-tail surfactants to a polyethyleneglycol-modified phospholipid. The use of these asymmetric triple-tail lipids is shown to result in a well-defined hierarchical layered assembly of helical peptides with a thermally reversible order–order or order–disorder transition (Article IV). Finally, simple anionic single-tail surfactants are combined with a diblock copolypeptide having one cationic block, leading to self-assembled structures at multiple length scales (Article V). A lamellar structure is observed at the block copolymer length scale, while inside both polypeptide blocks also a smaller periodic structure is formed due to the arrangement of the different secondary structures.

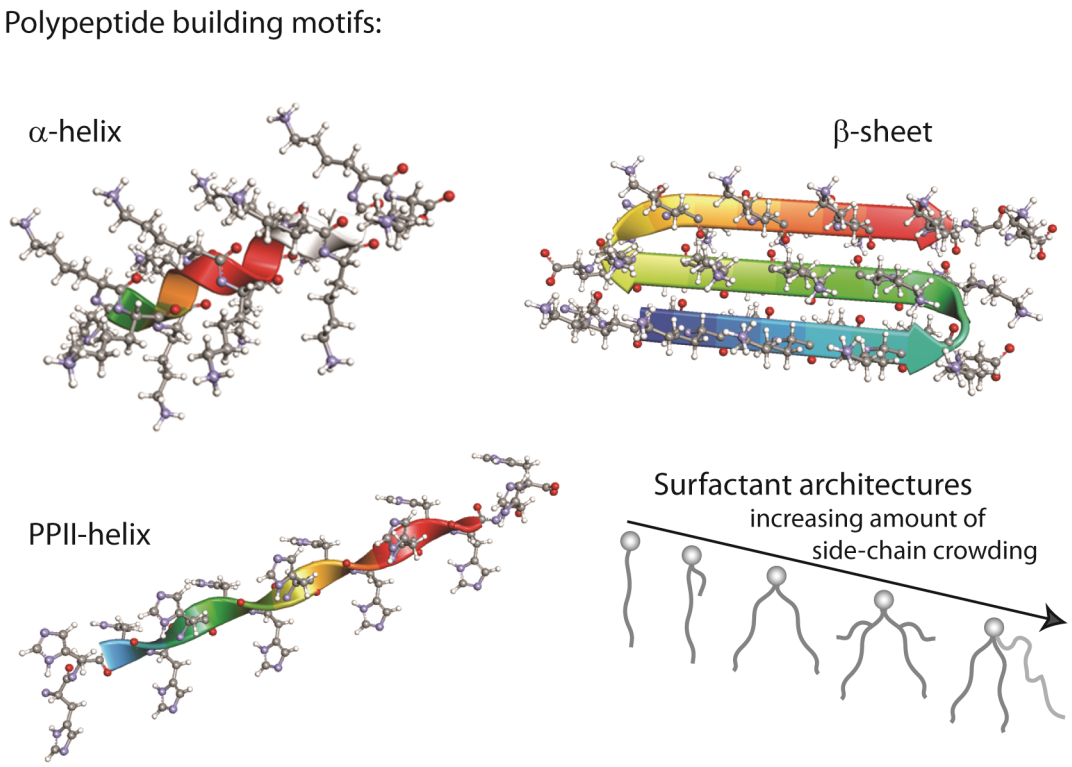

Figure 2. Polypeptide building motifs and surfactant architectures studied in this thesis.

2 Supramolecular Comb-Shaped Polypeptide–Surfactant Complexes

The chemical covalent bond in block copolymers, described in the Introduction, is not the only attractive interaction capable of creating the basis for polymeric self-assembly. Physical bonds, i.e. supramolecular interactions, can be strong enough to induce suitable attraction between repulsive moieties to hold the parts together and even to create linear supramolecular block copolymers as well.[40-42] On the other hand, as with covalent block copolymers, also supramolecular interactions enable other self-assembling molecular architectures besides the linear one and often with simpler preparation routes compared with the covalent analogues. One of the architectures is the supramolecular comb-shaped architecture, comparable to graft copolymers. There the surfactant molecules, which consist of a polar head group and a non-polar tail (typically an alkyl chain), are connected to a polymer backbone by physical interaction. The self-assembly is determined by the strength of attraction between the polar polymer backbone and the polar head group of the surfactant and repulsion of the non-polar alkyl tail from the polymer. The polymer–surfactant interactions can be for example ionic,[43-51] the strength of which is in the order of a covalent bond, coordination[52-56] or hydrogen bonds[57-60]. A typical self-assembled structure formed in polymer–surfactant systems in solid state is lamellar, as schematically illustrated in Figure 3, or hexagonal.[45, 61, 62] Also other more complex morphologies are reported, when the size and shape of the surfactant are varied.[43, 63, 64]

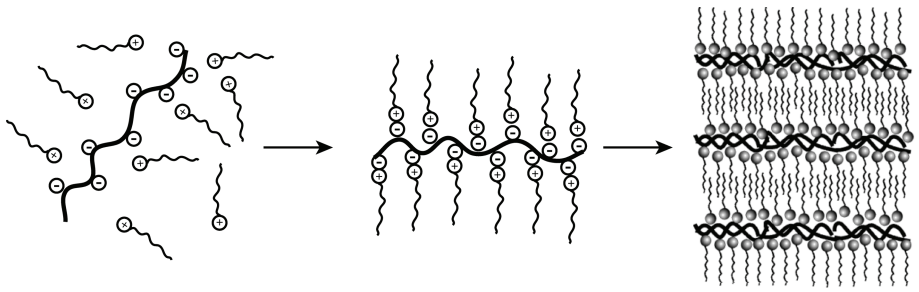

Figure 3. Schematic illustration of the self-assembled structure formation in comb-shaped polymer–surfactant complexes.

When the attractive interaction in comb-shaped material is electrostatic, the materials are called polyelectrolyte–surfactant complexes. These complexes are typically prepared from water from which they precipitate as near-stoichiometric complexes. It has been found that the complexation phenomenon is cooperative where one interacting surfactant molecule induces the interaction of the next molecule with the next possible repeating unit in the polymer (see Figure 3). In other words, the complexation takes place via a so-called zipper mechanism.[65-67]

In this thesis, the cooperative binding of surfactant molecules is used with polypeptides containing ionic amino acids in the peptide backbone. Whereas in traditional polyelectrolyte–surfactant complexes the polymer chains inside the polymer phase are in random order and the structure is determined by the attractive and repulsive interactions between the surfactant and the polymer, in polypeptide–surfactant complexes the formation of polypeptide secondary structures plays an additional role. Nevertheless, also in polypeptide–surfactant complexes the resulting self-assembly is often lamellar, where the polypeptide backbone has folded into, for example, β-sheet or α-helical conformation.[68-70] In hyperbranched polylysine–surfactant complexes, also other morphologies have been reported, but there the secondary structure of the peptide was suppressed due to the branching.[71] Additionally, for covalently modified homopolypeptide-based materials, various morphologies are found, often with additional features like liquid crystalline behavior.[33, 72-77] Here, we have investigated the possibilities offered by the easy preparation method of ionic complexation. It is known that the secondary structure formation is sensitive to the ambient conditions. By changing, for instance, the molecular shape of the surfactant, we show that it is possible to control the folding of the polypeptide backbone into a desired secondary structure and thereby affect the resulting self-assembled structure. The influence of different amino acids and variation in the surfactant architecture is studied in a systematic way.

2.1 Ionic polypeptide–surfactant complexes

In Articles I, II and III, the stoichiometric complexation of anionic surfactants with cationic homopolypeptides, poly-L-histidine (PHis) and poly-L-lysine (PLys)[†], was studied in order to find out the effect of, on the one hand, the specific amino acid forming the homopolypeptide and, on the other hand, the surfactant architecture to the resulting secondary structures and self-assemblies. Figure 4 shows the structural formulas for the polypeptides as well as for the surfactants, i.e. dodecylbenzenesulfonic acid (DBSA) and three different dialkyl phosphates, dioctyl (diC8), didodecyl (diC12) and bis(2-ethylhexyl) (diC2/6) phosphates.

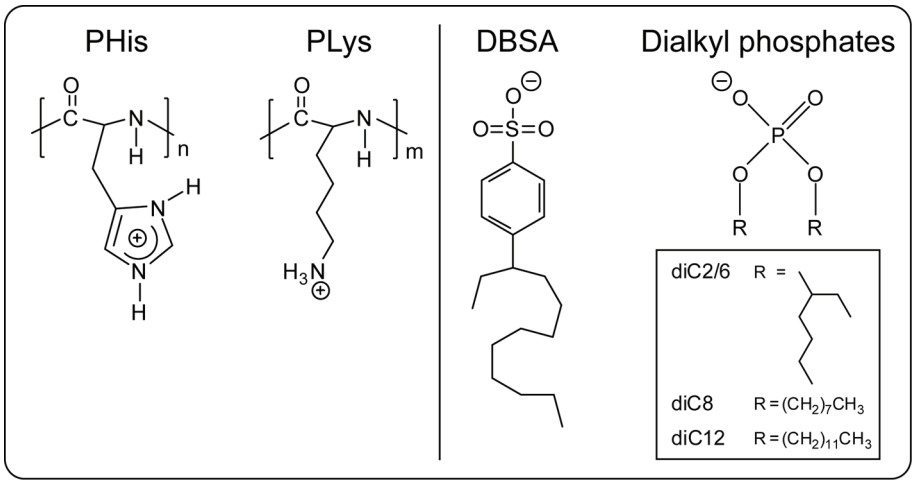

Figure 4. Structural formulas of the materials used in Articles I, II and III.

[†]In Article I poly-L-histidine has been denoted as PLH and in Articles II, III and V poly-L-lysine has been denoted as PLL, whereas in Article IV PHis and PLys are used, respectively. In this introduction part of the thesis, PHis and PLys are used for uniformity.

In Articles I and II, DBSA was complexed with PHis and PLys, respectively. Previously, the complexation of DBSA with different traditional polymers has been shown to lead to self-assembled structures, however, without any internal structure in the polymeric domains.[47, 49, 78-81] The small-angle x-ray studies (SAXS) for PHis(DBSA)$_{1.0}$ and PLys(DBSA)$_{1.0}$ prepared from aqueous solvent showed a lamellar morphology for both complexes (Figure 5a) with periodicities of 29 Å and 36 Å, respectively. However, from Fourier transform infrared (FTIR) spectroscopy measurements, the secondary structures of the complexes were found to be different. The amide I band in the range of 1700-1600 cm^{-1} is characteristic of C=O stretching and informs whether the carbonyl groups take part in hydrogen bonding. Two common secondary structures have amide I absorption bands at ~1655 cm^{-1} (α-helix) and 1632-1622 cm^{-1} with 1695-1685 cm^{-1} (antiparallel β-sheet). These absorption values are typical for strongly hydrogen-bonding carbonyls. In PHis(DBSA)$_{1.0}$ the polypeptide was found to have amide I absorption at a higher wavenumber, 1671 cm^{-1} (Figure 6a), indicating lack of hydrogen bonds. With additional circular dichroism (CD) measurements (see Article I Figure 2D) the secondary structure was assigned to PPII-type helix. PPII-helix is a left-handed helix without intramolecular hydrogen bonds (see Figure 2). It is typically found in polyproline polypeptides and has often been related to random coil structures. However, lately its role in short protein segment in processing (in silk) or in bringing elasticity (collagen and elastin) has been reported.[82-84]

On the other hand, PLys(DBSA)$_{1.0}$ showed amide I absorptions at 1694 and 1629 cm^{-1}, which stand for antiparallel β-sheet secondary structure (Figure 6a). This corresponds well with the previous reports of PLys complexes with linear alkyl tail surfactants.[69, 85] The previous studies on PLys–surfactant complexes have introduced the possible packing of surfactant alkyl tails, where the length of the alkyl tail plays a significant role. Typically, long crystallizing alkyl tails interdigitate, while shorter tails arrange in a tail-to-tail manner.[68, 85] In the DBSA complexes, the tail-to-tail arrangement of the surfactants is preferred, not only due to the length of the tail, but also because of the slight branching of the tail which suppresses crystallization. The quite large variation in the spacing of the two lamellar structures can be partly explained by the different molecular structure of the two amino acids as well as by the two very different secondary structures. Also, in the case of PHis complex, the possibility of slight alkyl tail interdigitation cannot be excluded, since the PPII-helix containing only three residues per round leads to a quite sparse incidence of DBSA molecules. However, the significance of the amino acids on the resulting structure was shown: complexation of DBSA with PHis leads to PPII-helix, while similar complexation of DBSA with PLys results in β-sheet conformation.

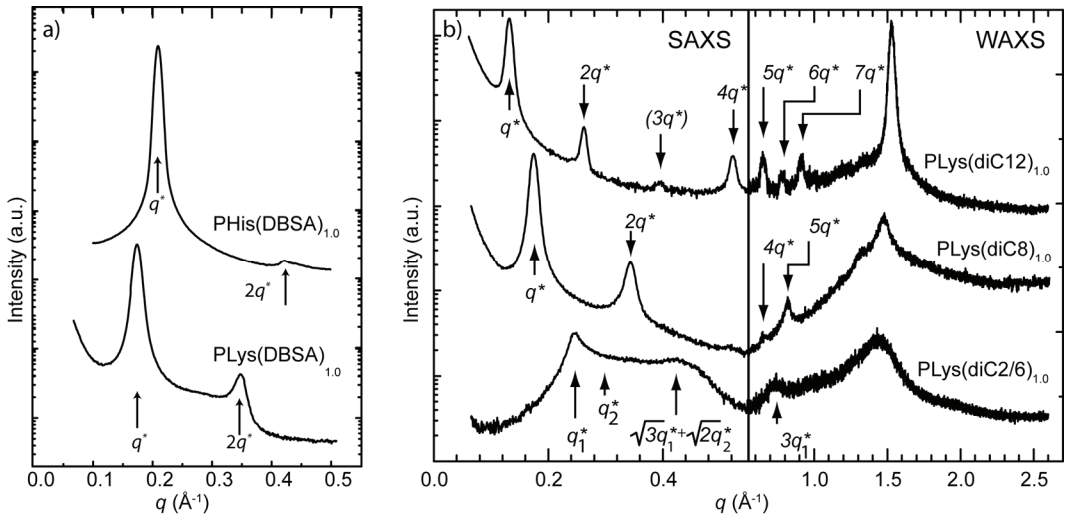

Figure 5. X-ray curves for a) PHis(DBSA)$_{1.0}$ and PLys(DBSA)$_{1.0}$ and b) PLys(diC12), PLys(diC8) and PLys(diC2/6) prepared from aqueous solution.

In addition to DBSA, which can be considered as a single-tail surfactant (with only slight branching), we wanted to study how the addition of another alkyl tail to the surfactant affects the complex behavior. For that, we took three double-tail phosphate surfactants with two linear alkyl tails, diC8 and diC12, and two branched alkyl tails, diC2/6 (see Figure 4 for the structures). FTIR measurements revealed β-sheet secondary structures for the two linear alkyl tail phosphates (amide I absorptions at 1695 and 1628 cm^{-1} in FTIR, Figure 6b), whereas in the complex with branched diC2/6 PLys was found to fold into an α-helix (absorption at 1657 cm^{-1}, Figure 6b). The transition from β-sheet to α-helical secondary structure was caused by the bulky nature of the diC2/6 compared with the linear tails in diC8 and diC12 because in the β-sheet conformation there is less space to accommodate the branched alkyl tails.

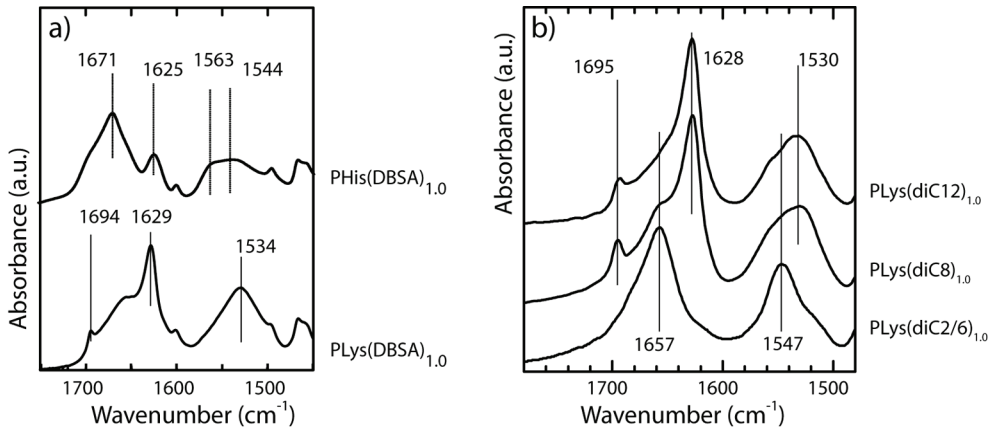

Figure 6. FTIR spectra in the amide band region for a) $PHis(DBSA)_{1.0}$ and $PLys(DBSA)_{1.0}$ and b) PLys(diC12), PLys(diC8) and PLys(diC2/6).

The self-assembled structures for the linear double alkyl tail complexes were found to be lamellar in SAXS measurements (Figure 5b) with a lamellar periodicity of 36 Å and 48 Å for $PLys(diC8)_{1.0}$ and $PLys(diC12)_{1.0}$, respectively. The lamellar spacing seems quite long in comparison with the above described $PLys(DBSA)_{1.0}$ complex, but can be explained by the increased amount of methylene units in the alkyl phase (12 methylenes in DBSA, 16 methylenes in diC8 and 24 methylenes in diC12). In addition, taking into account that the complexation ratio in all the complexes is stoichiometric, the surfactant architecture causes closer packing in lateral dimension for the double alkyl tail surfactant. This induces side chain stretching and thus increases the distance between the β-sheet layers. In $PLys(diC12)_{1.0}$ also the side chain crystallization had an effect on the periodicity. PLys(diC2/6) with α-helical secondary structure, in contrast, shows multiple broad and overlapping reflections in SAXS (Figure 5b). The fitting of Lorenzian peaks to the data suggested coexistence of two cylindrical morphologies as locally ordered domains without an overall defined order. The first reflections for hexagonal and tetragonal assembly are marked in Figure 5b as q_1* and q_2* corresponding to helix-to-helix distances of 29 Å and 22 Å, respectively.

Figure 7 shows schematic illustrations of the different structures in addition to some transmission electron microscopy (TEM) images of stoichiometric polypeptide–surfactant complexes in Articles I, II and III. The use of only slightly branched surfactant (DBSA) induced a lamellar morphology regardless of the used polypeptide. However, the secondary structure of the peptide changed from β-sheet in PLys to PPII-helix in PHis. On the other hand, the variations in the surfactant architecture resulted in increased layer periodicity in $PLys(diC8)_{1.0}$ and $PLys(diC12)_{1.0}$ compared with $PLys(DBSA)_{1.0}$. Increasing the branching in the surfactant in $PLys(diC2/6)_{1.0}$ caused crowding in the polypeptide–surfactant interface requiring curvature of the interface and thus induced the transition in the secondary structure from β-sheet to α-helix. This resulted in poor self-assembly having only local order with coexisting hexagonal and tetragonal morphologies.

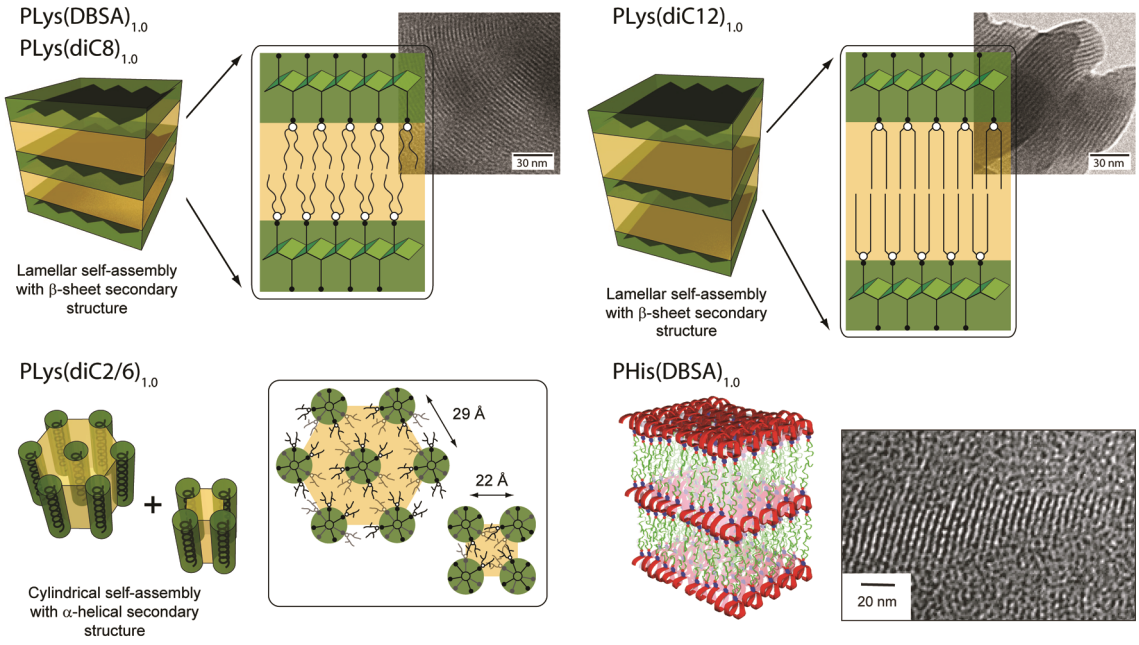

Figure 7. Schematic illustration of the structures found in stoichiometric polypeptide–surfactant complexes (Articles I, II and III). TEM images of $PLys(diC8)_{1.0}$, $PLys(diC12)_{1.0}$ and $PHis(DBSA)_{1.0}$.

2.2 Effect of stoichiometry on the self-assembly

Polypeptides composed of natural amino acids are solid materials at room temperature and they do not exhibit melting or glass transition to fluid-like state upon heating. This can become a problem if the materials are to be used in solid-state applications requiring processing like traditional polymers. Similar problems are encountered with other rigid, often conjugated polymers, and methods to overcome the challenge have been sought. One of the solutions reported for the problem is the addition of plasticizing small molecules, interacting through proton donation, which has been found to improve the processability properties as well as to induce self-assembly of the intractable polymers and even conductivity as in the case of polyaniline.[50, 51, 78, 79, 86-90] The stoichiometric complexation of polypeptides with surfactants described in the previous chapter can be considered a similar approach. It, however, did not yet result in plasticization required for melt state processing although it induced self-assembled structures and different solubility properties. Previously, it has been shown for other polymers that addition of amphiphilic molecules to ionic complexes is possible through hydrogen bonding, also in amounts over stoichiometry.[58, 80, 91-95] A similar method was used in Articles II and III for PLys–surfactant complexes to induce plasticization. Structural formulas for the complexes are shown in Figure 8. The surfactants used for hydrogen bonding were the same as the ionically interacting surfactants in stoichiometric complexes, i.e. DBSA (Article II) and dialkyl phosphates (Article III) from which $PLys(DBSA)_x$ and $PLys(diC8)_x$ complexes are described here.

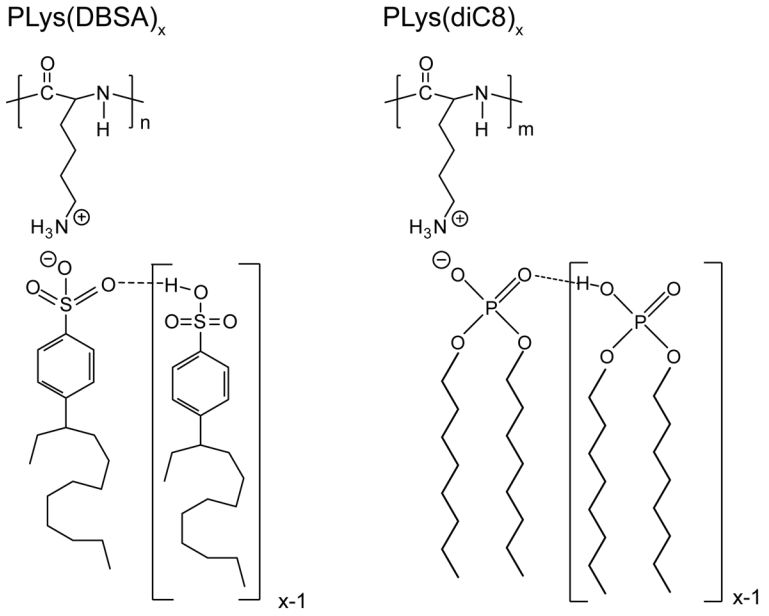

Figure 8. Structural formulas of the plasticized PLys complexes, PLys(DBSA)$_x$ and PLys(diC8)$_x$. The additional surfactants are suggested to bind through hydrogen bonds to the ionically bound surfactants.

To introduce additional surfactants to the materials, the ionic complexes were dissolved in organic solvents. For PLys(DBSA)$_x$, also a small amount of trifluoroacetic acid was used to open the insoluble β-sheets.[68] The acid forms of the surfactants were used for the addition. After solvent evaporation, interestingly, already the stoichiometric complexes showed changes in the self-assembly (SAXS in Figure 9), as well as in the secondary structures (FTIR in Figure 10). The dissolution in an organic solvent swells the alkyl tail phase to such extent that the β-sheet structure is not able to accommodate the tails anymore. Thus, curvature of the peptide–surfactant interface is required and the secondary structure is changed to α-helix. This is also shown in the self-assembly, which goes through a transition from lamellar to hexagonal morphology. However, the order is not very good in either of the described complexes (broad reflections in SAXS) and the materials still are hard and brittle.

As the amount of the surfactant is increased over the stoichiometric value, the secondary structure stays unchanged but the order is improved as evidenced by more distinct and narrower reflections in SAXS. Changes in the appearance of the materials are also observed. In Figure 9, the polarized optical micrographs (POM) of PLys(DBSA)$_x$ complexes are presented. Where the stoichiometric complex is hardly birefringent, the liquid crystalline nature of the material increases with the amount of added surfactant and the PLys(DBSA)$_{3.0}$ already shows clear liquid crystalline behavior. A corresponding change is observed also by naked eye, as shown for the PLys(diC8)$_x$ complexes (photographs in Figure 9). The stoichiometric complex is intractable, but as the amount of surfactant increases, the material becomes pliant and by PLys(diC8)$_{3.0}$ it is already soft shear-deformable.

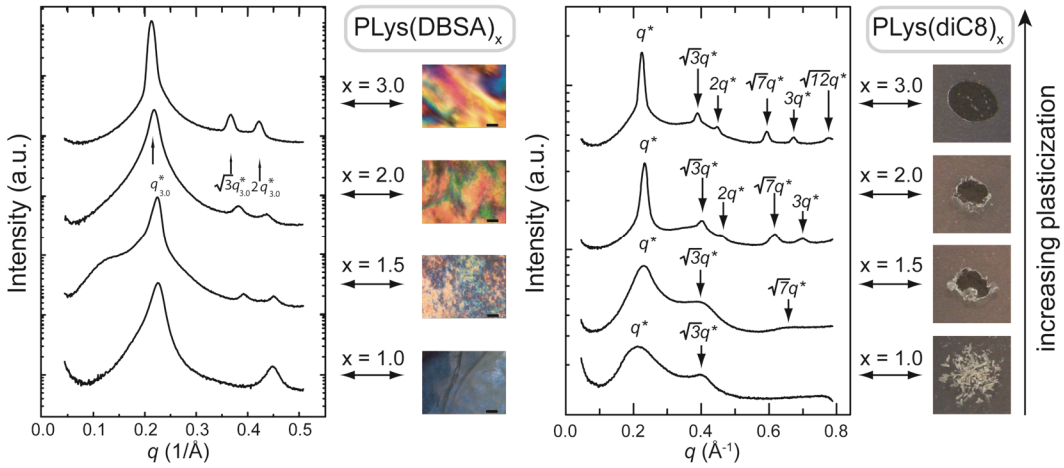

Figure 9. SAXS curves for the $PLys(DBSA)_x$ and $PLys(diC8)_x$ complexes showing improved order as the amount of plasticizing surfactant is increased. For $PLys(DBSA)_x$ POM images and for $PLys(diC8)_x$ photographs revealing the changes in the appearance of the materials with the amount of the surfactant are shown.

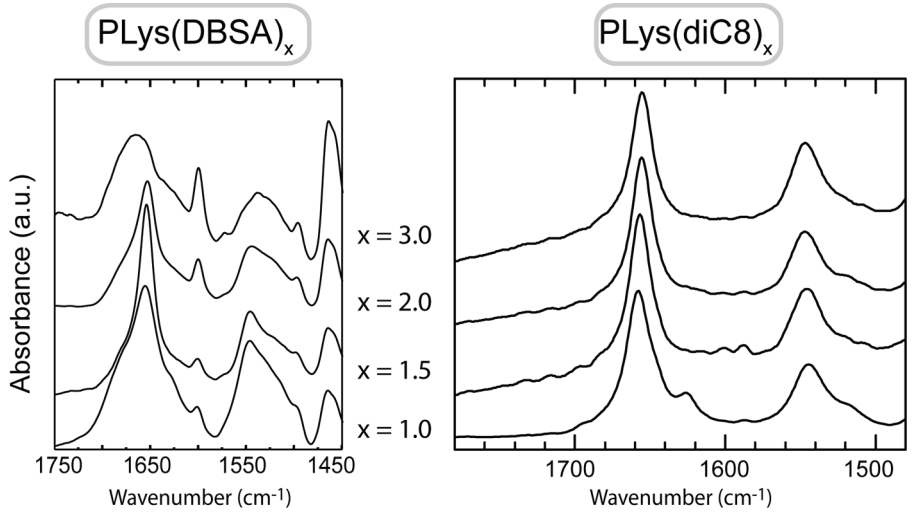

Figure 10. FTIR spectra in the amide band region showing the α-helical secondary structure for all the $PLys(DBSA)_x$ and $PLys(diC8)_x$ complexes, with the exception of $PLys(DBSA)_{3.0}$ where some suppression of helical conformation is observed.

The plasticization is a result of increased alkyl tail mobility. The added surfactant molecules are suggested to form hydrogen bonds with the ionically complexed surfactant oxygens, as presented in Figure 8. Although clear evidence on the hydrogen bonding could not be achieved due to overlapping absorption bands in FTIR, there is no reason to expect phase separation as the hydrogen bonded surfactants are similar to the ionically bonded ones. The POM micrographs support this hypothesis by showing no signs of phase separation. Indirect evidence of the interaction between the ionically bound and the additional surfactants was brought also by SAXS measurements. As can be seen from the SAXS data (Figure 9), the position of the first reflection for the complexes does not change dramatically as the amount of surfactant is increased, which means that the structure periodicity stays almost unchanged. In case of no

interaction, we would expect that the added DBSA or diC8 molecules would swell the surfactant phase, causing growth of the hexagonal periodicity. Schematic illustrations of the surfactant packing for PLys(diC8)$_{1.0}$ and PLys(diC8)$_{2.0}$ are presented in Figure 11a and b, respectively. As can be seen from the image, there is a considerable amount of space between the ionically bonded surfactants in the stoichiometric complex. In PLys(diC8)$_{2.0}$, the plasticizing diC8 has enough space for hydrogen bonds with the ionically bonded diC8 in the perimeter of the bottle-brush-like cylinders. At this point, it also has to be pointed out that for plasticization by hydrogen bonding surfactants, only amorphous surfactants are suitable. If the surfactants are to crystallize, a phase separation of the surfactants from the complex can be expected since the strength of crystallization is greater than that of hydrogen bonds.

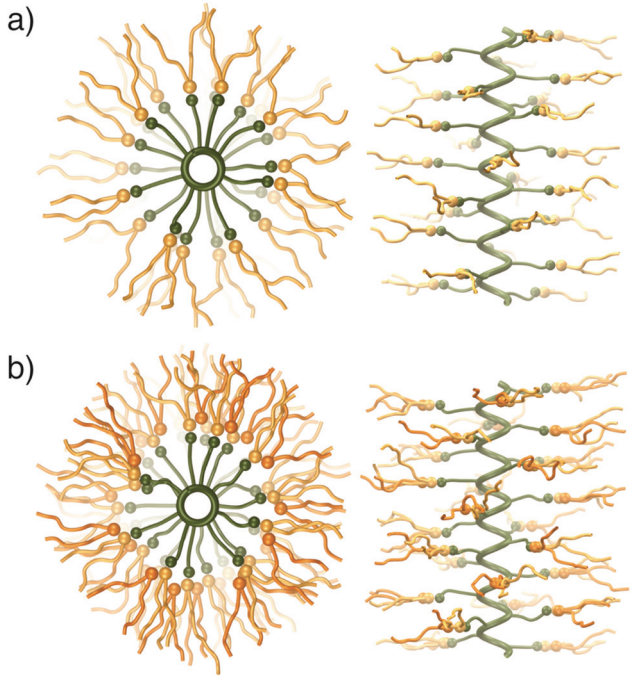

Figure 11. Schematic illustration of the suggested surfactant packing in the plasticized PLys(diC8)$_x$ complexes: a) x = 1.0 and b) x = 2.0.

2.3 Thermally induced phase transitions in polypeptide–surfactant complexes

Certain silks are known to have superior mechanical properties compared with other soft materials like Nylon and Kevlar, or even high-tensile steel. This results from a structure where hard β-sheet containing parts alternate with soft disordered domains.[96, 97] Silk has been an inspiration also for materials scientists to produce synthetic materials with comparable properties.[98-103] β-sheet structures are very hard to process and also silk worms and spiders have developed their mechanism to transform secondary structures during the spinning process. From materials science point of view, new concepts are needed to find materials, whose properties can be converted after the processing step with an external trigger, the easiest being temperature. As

the PLys complexes, described above, proved to be able to fold in different secondary structures, their response to change in temperature was also studied (Articles II and III). In addition, in Article I, the effect of temperature on PHis(DBSA)$_{1.0}$ was investigated for comparison with another polypeptide.

PHis(DBSA)$_{1.0}$ did not show any changes in the self-assembly as the complex was heated up to 220 °C. However, whereas the PLys complexes demonstrated a partial change in the self-assembly from one to another well-defined morphology after dissolution in an organic solvent, PHis(DBSA)$_{1.0}$ was observed to lose the structure after solvent treatment (Figure 12, for comparison with PHis(DBSA)$_{1.0}$ prepared from aqueous solution see Figure 5). This was puzzling since FTIR showed a sharp absorption for PPII-helix after solvent treatment (Figure 12). Upon heating, the secondary structure was lost but well-defined lamellar self-assembly was achieved as proven also by transmission electron microscopy (TEM) images in Figure 12. The reason for the behavior was deduced to result from the hydrogen bonding interaction of the surfactants with the polypeptide amides, which is possible due to the lack of intramolecular hydrogen bonds in PPII-helix. After casting from organic solvent, the DBSA sulfonates form hydrogen bonds with the amide NH-groups, which restricts the achievement of highly controlled lamellae formation although the secondary structure is well-defined. By heating, the secondary structure is deteriorated and the hydrogen bonds are opened, which gives enough flexibility for the complex to assemble in the lamellar morphology.

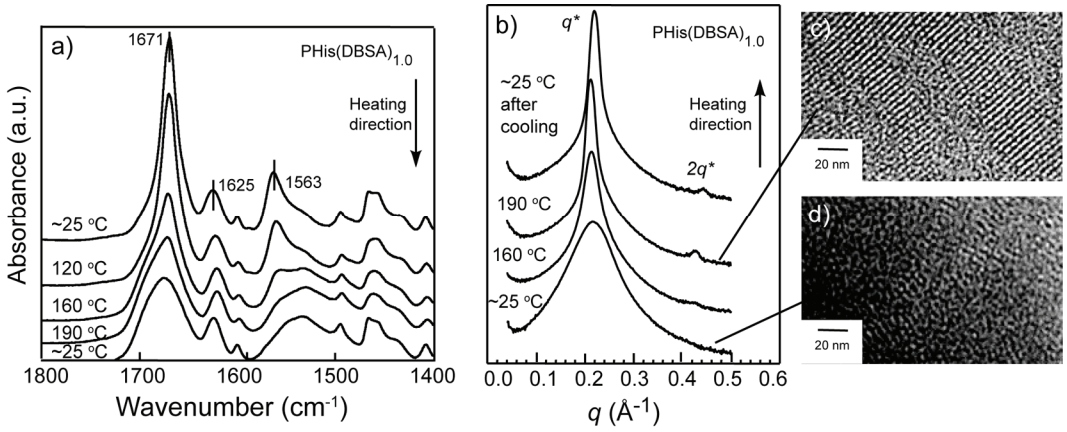

Figure 12. FTIR, SAXS and TEM results for PHis(DBSA)$_{1.0}$. After casting from organic solvent the complex shows improved ordering but a loss of secondary structure upon heating.

Different behavior is observed for PLys(DBSA)$_{1.0}$. As presented above, the complex forms a hexagonal cylindrical self-assembly with an α-helical secondary structure after casting from organic solvent. As the temperature is increased, changes are observed in the secondary structure as well as in the morphology (Figure 13). The α-helical secondary structure is gradually opened and partially turned into β-sheet. At the same time in SAXS, the reflection for hexagonal morphology ($q^*_{1.0}$) is found to decrease simultaneously with the arising reflection for lamellar self-assembly ($q^{**}_{1.0}$). The transition is, however, incomplete which leads to coexistence of lamellar and hexagonal structures. One explanation for the behavior resulting in mixed phases was reasoned to originate from the probability of the β-sheets starting to grow in several locations at the same time upon heating, and thus the α-helices can be trapped between the different β-sheet domains. Similar changes were observed also for the complexes with additional surfactants, PLys(DBSA)$_{1.5}$ and PLys(DBSA)$_{2.0}$, whereas in PLys(DBSA)$_{3.0}$ heating resulted in a loss of structure.

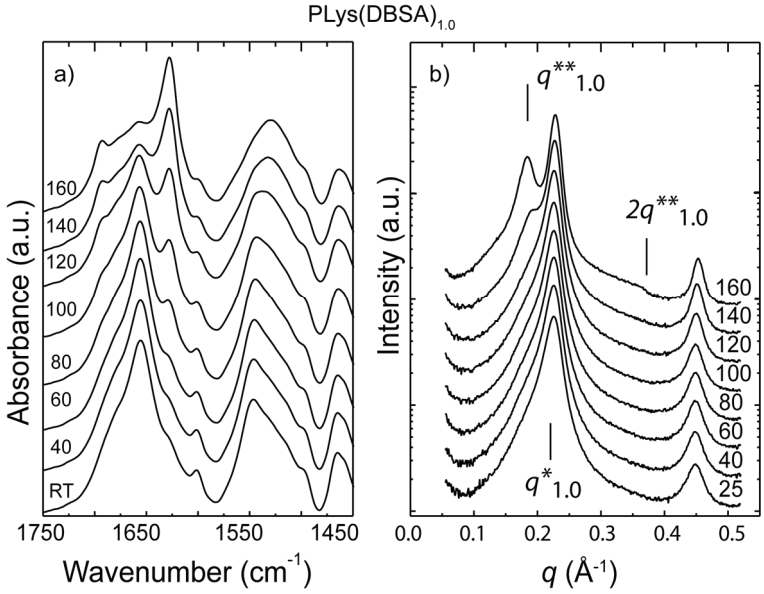

Figure 13. FTIR spectra and SAXS curves for $PLys(DBSA)_{1.0}$ cast from organic solvent showing the partial transition from α-helix to β-sheet and hexagonal to lamellar self-assembly.

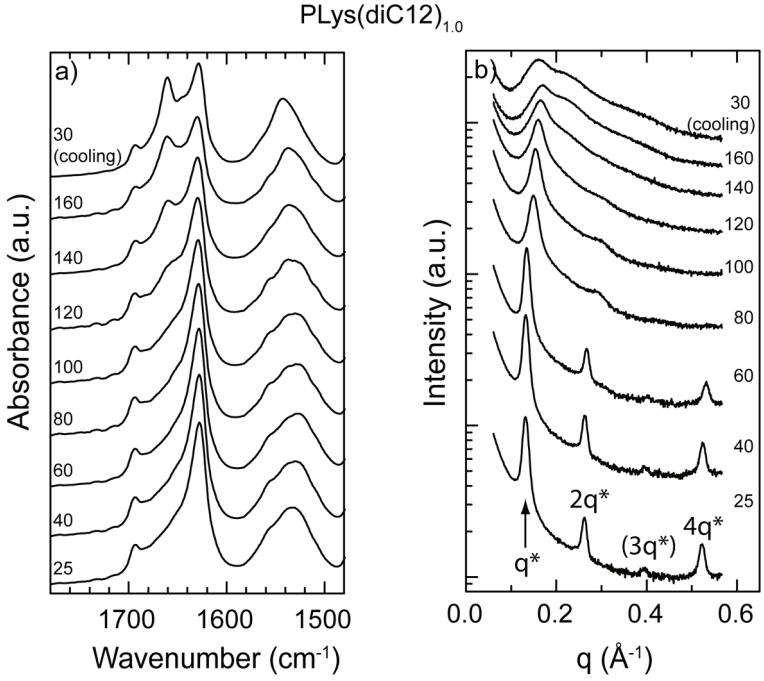

Figure 14. FTIR spectra for $PLys(diC12)_{1.0}$ prepared from aqueous solution showing a partial transition from β-sheet to α-helix as a function of temperature. Also SAXS curves show a change from lamellar structure, but no assignment of the high-temperature morphology can be given due to the broadness of the reflections.

After observing the partial transition in $PLys(DBSA)_x$ complexes with single alkyl tail, a similar treatment of the complexes with double alkyl tails was studied. Interestingly, $PLys(diC12)_{1.0}$ cast from organic solvent did not show any clear changes upon heating. Contrary to $PLys(DBSA)_{1.0}$, $PLys(diC12)_{1.0}$ prepared from aqueous solution shows a transition to the

reverse direction upon heating. In Figure 14 the FTIR and SAXS data are presented for the heating of PLys(diC12)$_{1.0}$ prepared from aqueous solution. FTIR shows a gradual change from β-sheet to α-helix as the temperature exceeds 100 °C. At the same time, the higher order reflections for lamellar self-assembly in SAXS disappear and q^* shifts to larger q-values. In addition, broad higher order reflections are observed. As in PLys(DBSA)$_x$, the transition is incomplete and no unambiguous assessment of the morphology can be made due to the broadness of the reflections.

A schematic presentation of the observed temperature induced transitions in the different complexes is shown Figure 15. The amino acid in the polypeptide is of great importance in the temperature behavior. Where the PLys(DBSA)$_x$ complexes show an interesting phase and secondary structure transition as a function of temperature, there are no big changes in the PHis(DBSA)$_{1.0}$ complex. On the other hand, the change of the surfactant from a single-tail one to a double-tail one turns the transition direction upside down. This is a proof of concept about the different possibilities in the polypeptide materials. By proper selection of the starting materials, desired properties can be achieved. However, detailed studies have to be made on the selected system in order to reach the ideally working conditions.

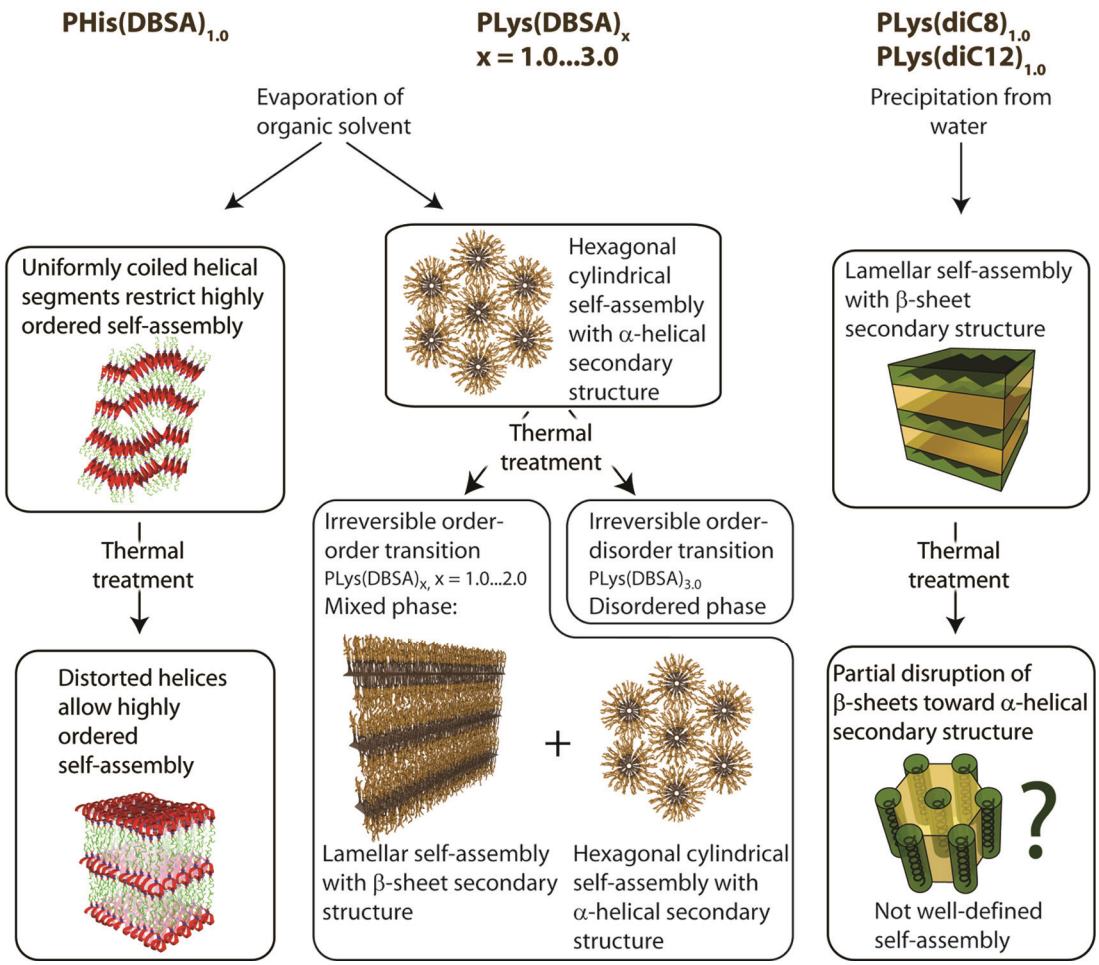

Figure 15. Schematic illustration of the transitions in different polypeptide–surfactant complexes observed upon heating.

3 Hierarchical Structures in Ionically Self-Assembled Polypeptide-Based Materials

There are multiple ways to obtain hierarchical structures, i.e. structures at different length scales, in polymer materials. Examples of molecular architectures enabling hierarchies are linear or branched ABC triblock or multiblock copolymers[6, 18, 104-106], rod–coil block copolymers[15, 107] or supramolecular comb–coil block copolymers.[58, 108-112] Replacement of one of the blocks with a polypeptide block in the above mentioned systems can give an additional variable to the self-assembly due to the secondary structure of the peptide block. Such behavior has been reported for hybrid block copolymers,[113-115] branched (miktoarm) copolymers,[116-118] as well as for supramolecular comb–coil block copolymers.[119, 120]
In Articles IV and V, the hierarchies have been created in polypeptide materials by two different approaches. In Article IV, rod-like polypeptides act as mesogens whose positional and orientational order is controlled with an asymmetric triple-tail lipid, leading to structures at different length scales and structural response to changes in temperature. In Article V, hierarchy is generated by an addition of different surfactants to one of the blocks in a diblock copolypeptide, resulting in rod–comb molecules with structure-*and*-structure-*within*-structure arrangement of the molecules.

3.1 Lipid induced hierarchy

In Article IV, the study on the effect of surfactant architecture on ionic self-assembly with polypeptides was developed by increasing the number of tails in the surfactant to three. The surfactants were PEG-modified natural lipids, whose structural formulas together with a schematic illustration are presented in Figure 16. There has been considerable interest in hydrophilic modification of lipids by PEG chains resulting in PEG-lipids, especially due to their properties as drug carriers.[121] On the other hand, architecturally they can be considered as low molecular weight analogies to miktoarm star copolymers which allow novel polymeric self-assemblies, such as quasicrystalline tiling patterns.[122] We studied the effect of these A_2B miktoarm-like phospholipids on the supramolecular assembly with polypeptides and found hierarchical order. In addition to the structural changes in the surfactant, the variation in cationic polypeptides was increased from PLys and PHis to poly-L-arginine (PArg), the structures of which are shown in Figure 16b, c and d, respectively.

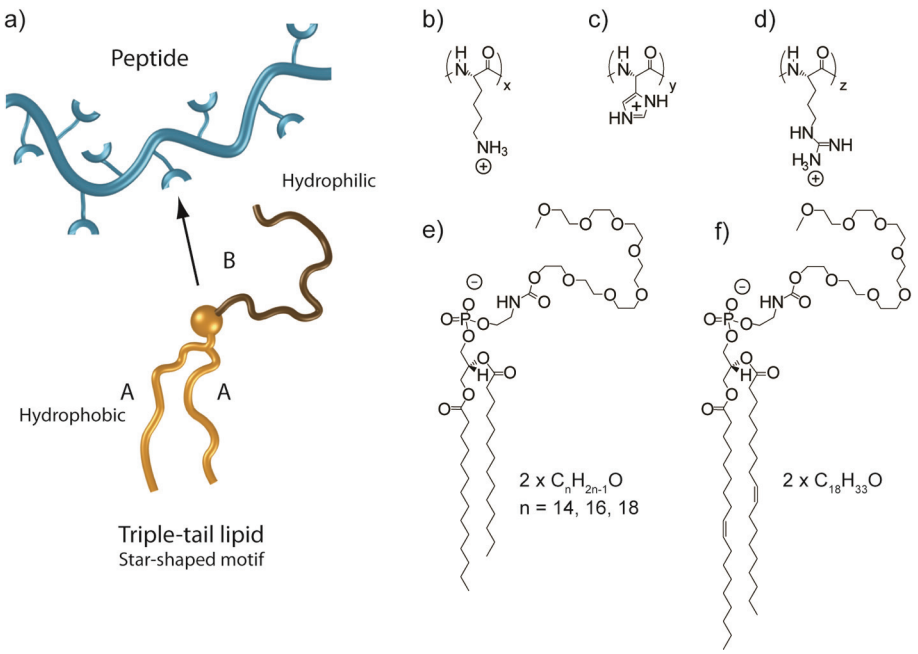

Figure 16. a) A schematic illustration of the complex formation. Structural formulas of the starting materials: b) PLys, c) PHis, c) PArg, e) PEGylated phospholipid with three different saturated alkyl tail lengths (diC14-PEG, diC16-PEG, and diC18-PEG), and f) PEGylated phospholipid with unsaturated alkyl tails (diC18*-PEG). The components are shown in their charged form.

The stoichiometric complexes were precipitated from water or water/isopropanol solutions. The as-formed structures were studied with SAXS, TEM and FTIR. For PLys(diC14-PEG) complex, the data for the determination of the structure is gathered in Figure 17. From the FTIR data, α-helical secondary structure was deduced based on the amide absorptions at 1651 cm^{-1} and 1547 cm^{-1}. The TEM image showed a layered overall structure with a smaller structure inside the layers. From the image, we made an assumption of an oblique structure and fitted [123, 124] the parameters of such a structure to the SAXS data. The indices for the Miller planes in an oblique columnar structure with lattice parameters of a = 45 Å, b = 28 Å and γ = 107° are marked in the SAXS curve in Figure 17b. In addition to the reflections observed in SAXS, the fitted data corresponded well also with the FFT taken from the TEM micrograph as shown in Table 1, where the experimental and theoretical values for the structure are given.

This was the first time that an oblique arrangement of helices is reported in a synthetic polypeptide–lipid complex. A schematic illustration of the structure is presented in Figure 17d. The hydrophilic cylindrical helices (blue) form layers with the PEG tails (brown) of the triple-tail lipid filling in the voids between the helices. These hydrophilic layers are separated by lamellae formed by the hydrophobic lipid alkyl tails (yellow).

Comparable results for layered helical structures with positional order, i.e. 2D registry between the helices in different layers, have been previously reported for DNA–lipid complexes, where non-ionic helper lipids were used for balancing the structure.[125-127] A similar approach has been used for genetically prepared $(A_3E)_n$ peptide–lipid complexes, where the correlation of monodisperse α-helices with each other was controlled with the aid of a helper lipid.[128] However, for synthetic polypeptide complexes the registry between the helices has not been previously reported.

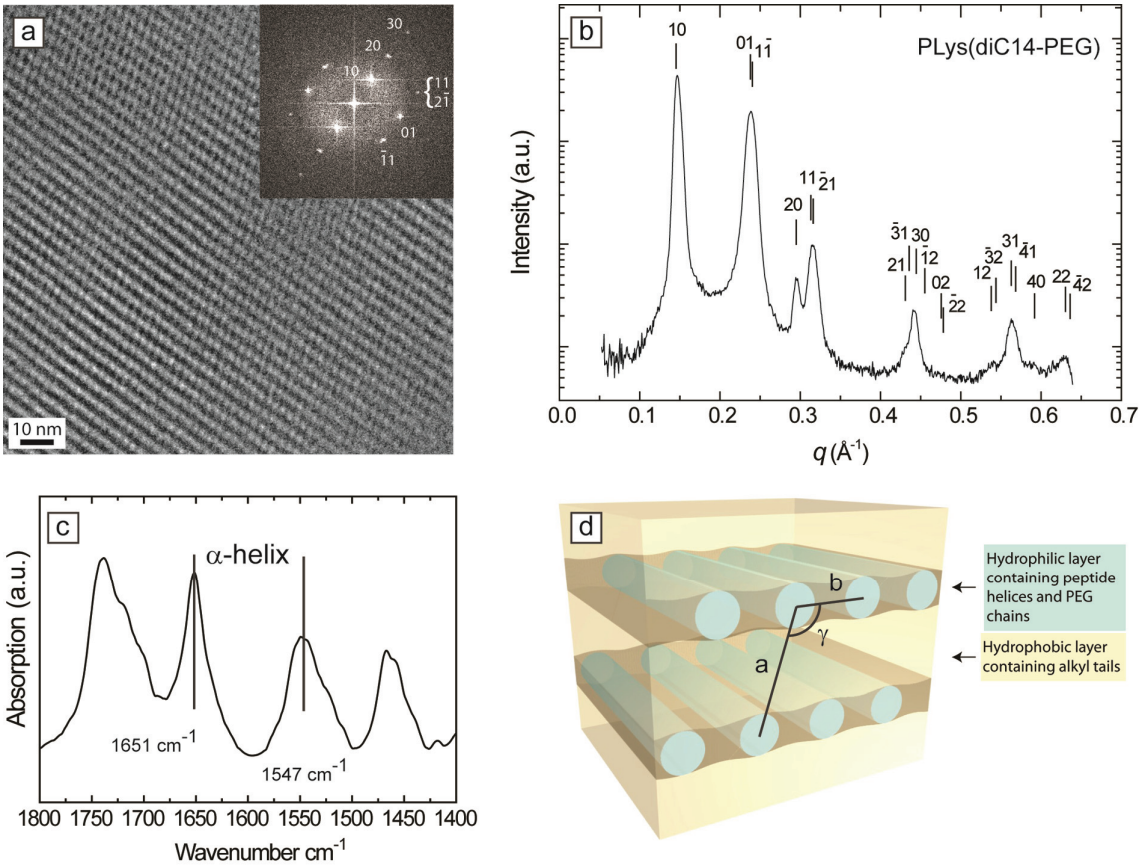

Figure 17. Structural analysis of PLys(diC14-PEG). a) TEM micrograph showing highly ordered lamellar periodicity with an internal structure within the lamellae. Inset: Fast Fourier transform (FFT) of the TEM image. b) SAXS curve with Miller indices of the fitted oblique structure. c) FTIR spectrum showing the amide I and II bands which correspond to an α–helical secondary structure. d) Schematic illustration on how the hydrophilic cylindrical helices (blue) are correlated with each other in the three-phase layered structure. The PEG domains (brown) and hydrophobic alkyl tail layers (yellow), as well as the lattice parameters *a*, *b* and *γ* are shown.

Table 1. Assignment of the observed SAXS and TEM reflections for PLys(diC14-PEG).

h k	q_{SAXS} (Å^{-1})	q_{TEM} (Å^{-1})	$q_{theor.}$ (Å^{-1})	φ_{TEM}	$\varphi_{theor.}$
1 0	0.147	0.152	0.148	0°	0°
$\bar{1}$ 1	0.238	0.242	0.240	104°	107°
0 1	0.238	0.248	0.238	69°	71°
2 0	0.295	0.305	0.296	0°	0°
1 $\bar{2}$	0.315	0.336	0.320	44°	45°
1 1	0.315	0.336	0.315	44°	46°
3 0	0.442	0.460	0.444	0°	0°

In our work, the applicability of an asymmetric triple-tail lipid to create hierarchical order was extended from one cationic polypeptide (PLys) to two other polypeptides (PArg and PHis) as well as from one lipid alkyl chain length to two other chain lengths.
Figure 18 shows the SAXS curves for complexes with alkyl tails extended with two methylene units, i.e. PLys(diC16-PEG), PArg(diC16-PEG) and PHis(diC16-PEG). Also TEM micrographs with FFTs as insets for the latter two are presented. The fitting of the oblique structure to the SAXS data resulted in lattice parameters that correspond well with observed reflections. The lattice parameters for the different complexes are gathered in Table 2.

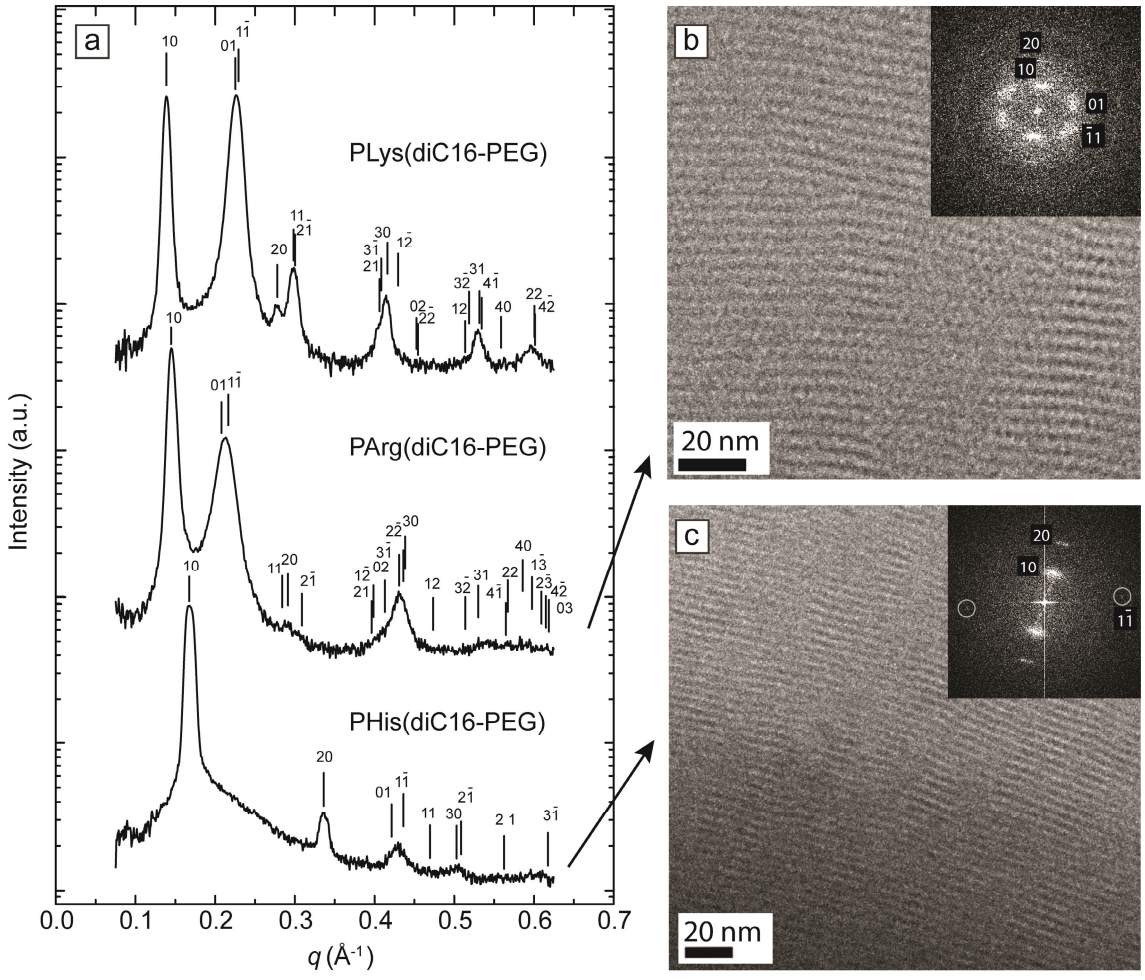

Figure 18. a) SAXS curves of PLys(diC16-PEG), PArg(diC16-PEG) and PHis(diC16-PEG) at room temperature. TEM micrographs of b) PArg(diC16-PEG) and c) PHis(diC16-PEG) with FFT patterns as insets. The Miller indices for oblique structure are shown for both the SAXS and the FFT patterns.

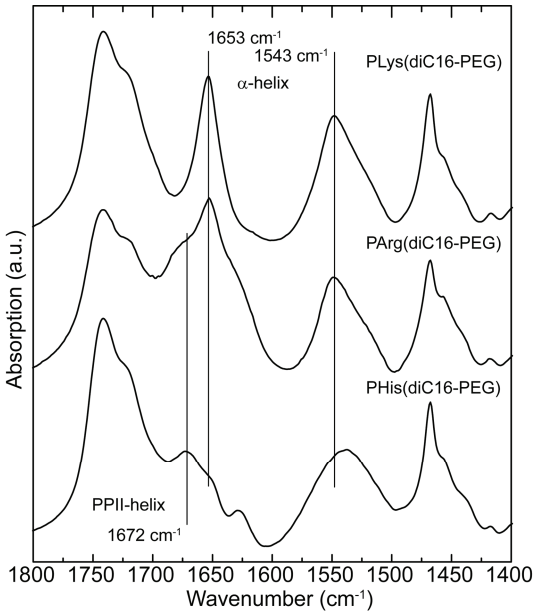

Figure 19. FTIR spectra of PLys(diC16-PEG), PArg(diC16-PEG) and PHis(diC16-PEG) in the amide band region showing α-helical and PPII-type helical structures for the complexes.

Table 2. The lattice parameters *a*, *b* and γ for the peptide–lipid complexes and the orthogonal interlayer distance *d* for the layered structure (see also Figure 21 for definitions).

	a (Å)	*b* (Å)	γ (°)	*d* (Å)
PLys(diC14-PEG)	45	28	107°	43
PLys(diC16-PEG)	47	29	108°	45
PLys(diC18-PEG)	50	26	105°	48
PArg(diC16-PEG)	45	32	105°	43
PHis(diC16-PEG)	38	15	96°	38

The large differences in the lattice parameters between the different polypeptide(diC16-PEG) complexes can be explained by the different size of the amino acids, but also by different helical structures as revealed by FTIR (Figure 19). For PLys(diC16-PEG) and PArg(diC16-PEG) the secondary structure was α-helical as concluded from the absorptions at 1653 and 1543 cm^{-1}. PHis(diC16-PEG), however, showed an absorption at 1672 cm^{-1}, which already earlier was proven to originate from a PPII-type helical structure. In a PPII-helix there are 3 repeating units per one round of the helix with a rise of 3.1 Å per residue in comparison with 3.6 units per round and 1.5 Å rise per residue in α-helix. This induces different demands for the packing in order to gain as uniform density as possible all over the material and results in different lattice parameters. In addition, the different secondary structures in the polypeptides affected the

crystallization of the lipid alkyl tails. The melting temperatures of the alkyl tails were measured with differential scanning calorimetry (DSC). PHis(diC16-PEG) showed melting at 25 °C while in PLys(diC16-PEG) with the same lipid the alkyl tail crystallites melted at 44 °C, the latter being very close to the melting temperature of the pure lipid. This would imply that the packing of the lipids is more perfect in PLys complexes, i.e. the α-helical secondary structure offers a better "scaffold" for the packing.

The crystallization of the lipid alkyl tails was also found to be crucial for the layered structure. For the complex PLys(diC18*-PEG) with double bonds in the alkyl tails, no crystallization temperature was observed, i.e. the alkyl chains stayed amorphous in all temperatures used in the study. This directly affected the self-assembly: the structure was found to be hexagonal columnar, not oblique. For the other complexes, SAXS was measured upon heating the complexes above the melting temperature of the saturated alkyl tails and for PLys and PArg complexes an order–order transition from oblique to hexagonal columnar structure was observed as revealed by the change in reflections to a ratio of 1:√3:2 at 80 °C (Figure 20). Also for PHis(diC16-PEG) a transition was found, but due to the lack of multiple reflections at high temperatures, no assignment of the structure could be made. The broadness of the first reflection suggests that even a disordered state cannot be excluded. The original reflection pattern for oblique columnar structure returned after cooling the complexes back to room temperature.

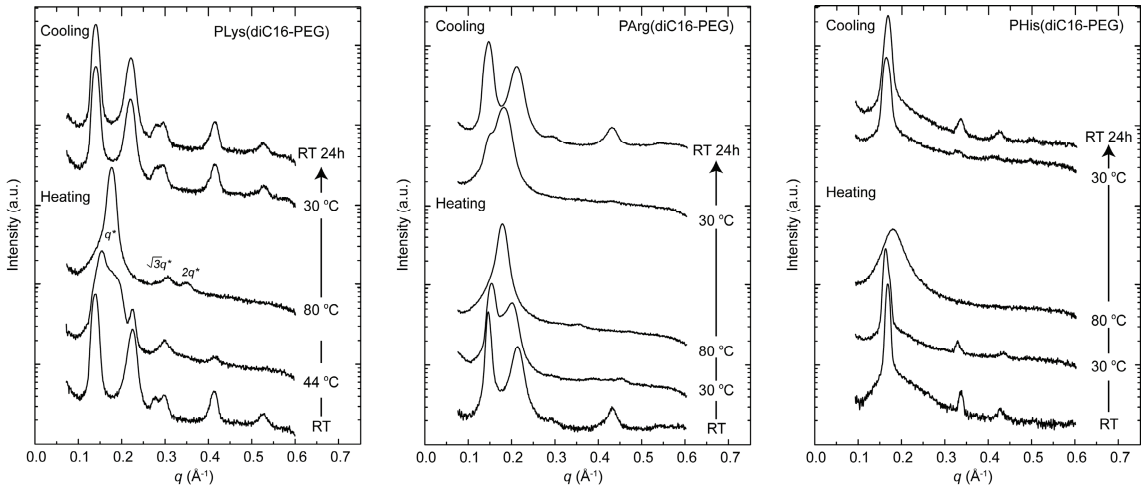

Figure 20. SAXS heating and cooling curves for PLys(diC16-PEG), PArg(diC16-PEG) and PHis(diC16-PEG) showing the reversible order–order transition from oblique to hexagonal structure for PLys, and PArg complexes and the reversible transition for the PHis complex.

From the DSC measurements for PLys complexes with three different lipid alkyl tail lengths, we were able to estimate the degree of crystallization in the side chains.[75, 129, 130] Based on the measurements, the saturated alkyl tails in the PLys complexes contain 6-7 amorphous carbons per tail while the rest of the methylene units participate in crystallization. Also the interdigitation and direction of the crystallization normal to the layer direction was deduced from the combination of DSC and SAXS results, the latter showing the growth of the layer periodicity (see Table 2). Linear growth by the length of two crystallizing methylene units is observed as the alkyl tail length is increased from 14 to 16 and from 16 to 18 carbons.

A schematic illustration of the hierarchical[‡] layered structure observed at room temperature and the hexagonal columnar structure is shown in Figure 21. At room temperature, the A_2B lipid forms a three-phase junction point with the polypeptide chains. The helical polypeptide backbones (blue) are confined in the hydrophilic layers, where the PEG tails (brown) control the intralayer distance between the helices. In addition, the hydrophobic lipid alkyl tails (yellow) determine the distance between the layers. The lipid alkyl tails are partly crystalline, which arranges the complex to a layered structure instead of the more symmetric hexagonal cylindrical morphology. Upon heating, the locking created by crystalline alkyl tails opens and a transition to the hexagonal columnar structure takes place. The amorphous alkyl tails require more lateral space, and since the crystallites are no longer present, no direction of the tails is preferred over the other. However, as the temperature is decreased again, the oblique arrangement of the helices returns, underlining the power of crystallizing alkyl tails in the structure formation.

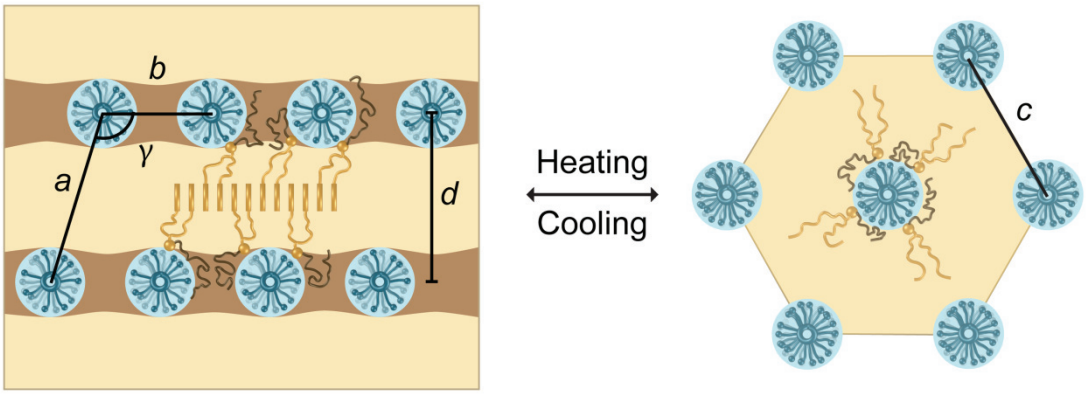

Figure 21. A schematic presentation of the self-assembly of polypeptide–A_2B lipid complexes with saturated alkyl tails at room temperature (on the left). The alkyl tails control the separation of the peptide-containing hydrophilic layers and the PEG tails control the separation of the helices within the layers. The lattice parameters *a*, *b* and *γ* define the oblique structure. Also, the orthogonal interlayer distance *d* is denoted in the figure. Upon heating the structure is transformed to hexagonal columnar packing (on the right) because of the increased space requirement of the melting side chains and symmetric interactions due to absence of crystallization. The helix-to-helix distance is denoted by *c*. The order–order transition is reversible.

We believe that the hydrophilic PEG tail is a crucial element in the well-defined hierarchical self-assembly. The non-crystalline PEG acts as a plasticizer and spacer within the hydrophilic nanodomains, making the packing of the rigid α-helices easier and more defined. It also balances the density of the hydrophobic and hydrophilic phases by filling in the voids between the α-helices. Previously, stoichiometric complexes of helical peptides with crystallizing surfactants or natural lipids have resulted in lamellar structure with no correlation between the helices in different layers.[69, 70] In other words, the excellent positional correlation of the α-helix layers found in this study is believed to result from the interdigitation of the crystalline alkyl tails as well as the presence of the PEG tail in the lipid. The relatively small amount of crystallizing methyl units in the alkyl tails allows the material to be flexible enough to find the best possible space filling despite the rapid preparation by precipitation.

[‡]We use here the terms hierarchical or structures at different length scales. However, the question can be raised, whether the structure could be called crystal, due to the crystallization of the alkyl tails and 2D correlation of the well-defined helices. Nevertheless, the PEG tails and part of the alkyl tails remain amorphous, which disagrees with the definition of a crystal.

3.2 *Block copolypeptide–surfactant induced hierarchy*

Interest in synthetically prepared block copolymers with polypeptide blocks has grown enormously, especially after the development of synthetic routes that allow narrow polydispersities.[131-134] Lots of studies have been made on the solid state properties of block copolymers where one block is peptidic inducing hierarchy to the material.[13, 113-115, 135, 136] Also pure block copolypeptides have gained interest[137, 138], and lately even block copolypeptides with complex architectures have been reported.[116, 117]

In Article V the complexity of the hierarchical polypeptide materials was taken one step further from Articles I-IV. Instead of using homopolypeptides, a diblock copolypeptide poly(γ-benzyl-L-glutamate)-block-poly(L-lysine) (PBLG-*b*-PLys) was studied for ionic complexation with two surfactants, DBSA and dodecanesulfonic acid (DSA), see Figure 22. Unlike in the homopolypeptide–surfactant complexes that were prepared from aqueous solutions by precipitation, the block copolypeptide complexes were made by acid-base proton transfer reaction in an organic solvent due to limited solubility in water. Stoichiometric amount of surfactants per PLys repeating unit were used.

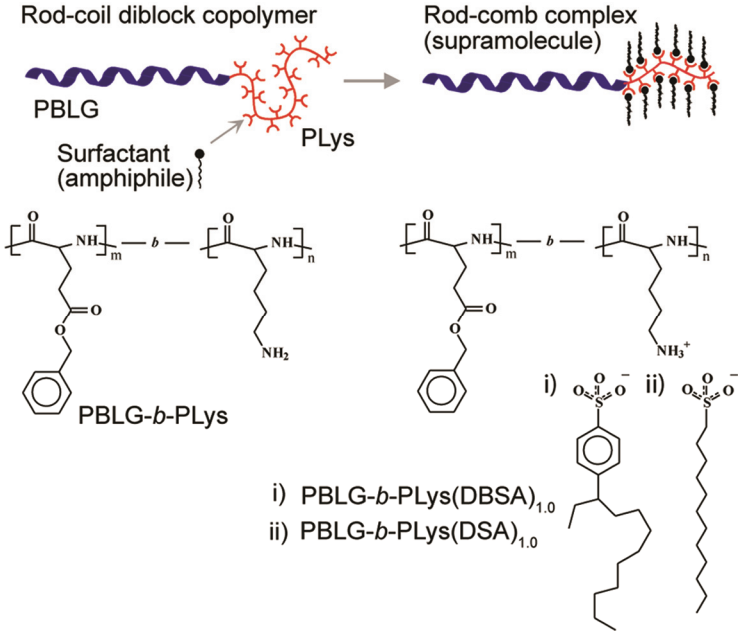

Figure 22. Schematic presentation of the diblock copolypeptide–surfactant complexes and the structural formulas for the materials.

The structural hierarchy at the diblock copolypeptide and the surfactant length scales, i.e., at tens of nanometers and a few nanometers length scales, were studied by TEM and SAXS, supported by FTIR and POM. Figure 23 shows the TEM micrographs of PBLG-*b*-PLys, PBLG-*b*-PLys(DBSA)$_{1.0}$, and PBLG-*b*-PLys(DSA)$_{1.0}$. In all cases, a well-defined lamellar structure is observed at the diblock copolypeptide length scale. Smaller surfactant-scale structures were not resolved in TEM. However, the SAXS measurements showed reflections at both diblock copolypeptide and surfactant length scales. In combination of TEM and SAXS results, we were able to assign the self-assembly on the block copolypeptide level lamellar for pure block copolypeptide as well as for both complexes. The lamellar periodicity varied from 27 nm in pure PBLG-*b*-PLys to 29 nm and 33 nm in PBLG-*b*-PLys(DBSA)$_{1.0}$ and PBLG-*b*-PLys(DSA)$_{1.0}$, respectively. The increase in periodicity was due to structural changes in the PLys-surfactant block upon complexation as described below.

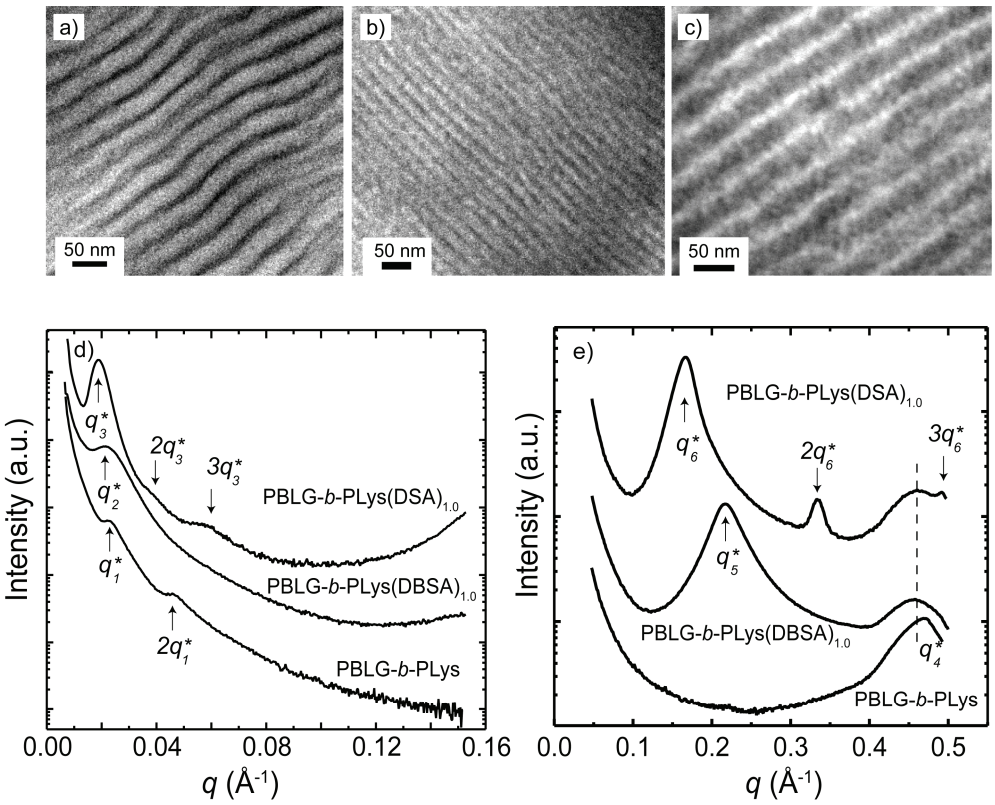

Figure 23. Top: TEM micrographs of diblock copolypeptide–surfactant complexes for (a) PBLG-*b*-PLys, (b) PBLG-*b*-PLys(DBSA)$_{1.0}$, and (c) PBLG-*b*-PLys(DSA)$_{1.0}$. In (a) the PBLG block appears light and in (b) and (c) dark because of the different staining requirements. Bottom: SAXS graphs showing the structures (d) at the diblock copolypeptide length scale and (e) at the surfactant length scale.

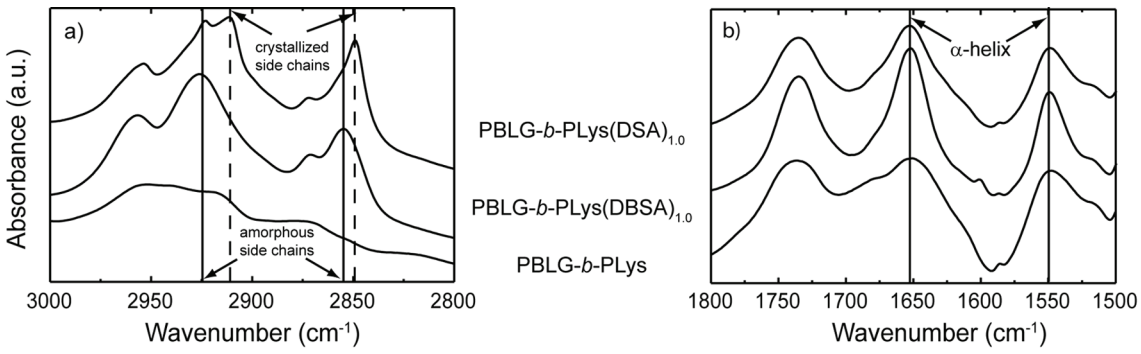

Figure 24. FTIR spectra of PBLG-*b*-PLys, PBLG-*b*-PLys(DBSA)$_{1.0}$, and PBLG-*b*-PLys(DSA)$_{1.0}$: a) C-H stretching range: amorphous branched dodecyl chains and partly crystallized *n*-dodecyl chains. b) Amide band range: characteristic absorptions for α-helix are observed for both complexes and the pure block copolypeptide, although for the latter some random coil characteristics attributed to the PLys block are suggested due to the shoulders around the α-helical absorption.

More pronounced differences between the samples came up at the surfactant length scale. In the pure PBLG-*b*-PLys, only relatively broad reflection at q_4^* = 0.47 Å^{-1} corresponding to periodicity of 1.3 nm was visible in SAXS for large measuring angles. This has previously been attributed to a hexagonal arrangement of PBLG helices.[138, 139] A similar packing of PBLG helices was observed also for the complexes but in addition there were other reflections originating from the PLys–surfactant block. In PBLG-*b*-PLys(DSA)$_{1.0}$ a lamellar organization with periodicity of 3.7 nm was revealed by three reflections in ratio 1:2:3. In PBLG-*b*-PLys(DBSA)$_{1.0}$, on the other hand, a pronounced reflection at q_5^* = 0.22 Å^{-1} was observed, corresponding to a structure periodicity of 2.9 nm, but due to the lack of higher order reflections, no direct assignment of the morphology could be given. Nevertheless, a hexagonal arrangement of the PLys helices was suggested after taking into account the previous results for homoPLys(DBSA) complexes. In addition, the FTIR results (Figure 24b) indicating α-helical secondary structures for both blocks in PBLG-*b*-PLys(DBSA)$_{1.0}$ as well as the amorphous nature of the alkyl chains supported the suggestion. From the literature and our previous results, we know that in case of helical secondary structure, crystallization of alkyl tails is often needed for the other typical alternative, i.e. layered morphology. Also here, PBLG-*b*-PLys(DSA)$_{1.0}$ shows a lamellar morphology in SAXS for the PLys(DSA)$_{1.0}$ block although the secondary structure is α-helix, and from the FTIR at least partial crystallization of the alkyl tails can be deduced (Figure 24a). Additionally, in POM (Figure 25) the appearance of the materials is different. PBLG-*b*-PLys and PBLG-*b*-PLys(DSA)$_{1.0}$ show birefringent but crystalline pattern in POM, whereas a clear plasticization is observed in the case of DBSA complexation resulting in liquid crystalline behavior.

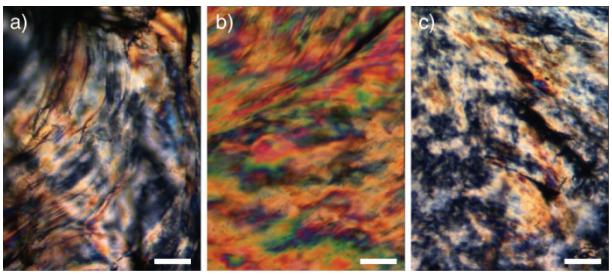

Figure 25. Polarized optical micrographs of the supramolecular complexes and pure polymer: a) PBLG-*b*-PLys, b) PBLG-*b*-PLys-(DBSA)$_{1.0}$, and c) PBLG-*b*-PLys(DSA)$_{1.0}$. The scale bar is 50 μm.

From the results above we can suggest hierarchical structures for all the studied materials: hexagonal-*in*-lamellar for PBLG-*b*-PLys, hexagonal-*and*-lamellar-*in*-lamellar for PBLG-*b*-PLys(DSA)$_{1.0}$ and hexagonal-*and*-hexagonal-*in*-lamellar for PBLG-*b*-PLys(DBSA)$_{1.0}$. A schematic presentation of the structures is given in Figure 26. The structural hierarchies were achieved via interplay between the diblock copolypeptide self-assembly at the tens of nanometers length scale, the polyelectrolyte–surfactant self-assembly with an additional effect from the PLys secondary structure at an order of magnitude smaller length scale, and the packing of the rod-like PBLG helices with 1.3 nm periodicity. By the addition of surfactant molecules to the PLys block we were able to tune the lamellar periodicity of the block copolypeptide self-assembly. In addition, the morphology of the PLys-surfactant block was controlled, i.e. either lamellar or hexagonal arrangement of the PLys α-helices were observed depending on the capability of the surfactant to crystallize.

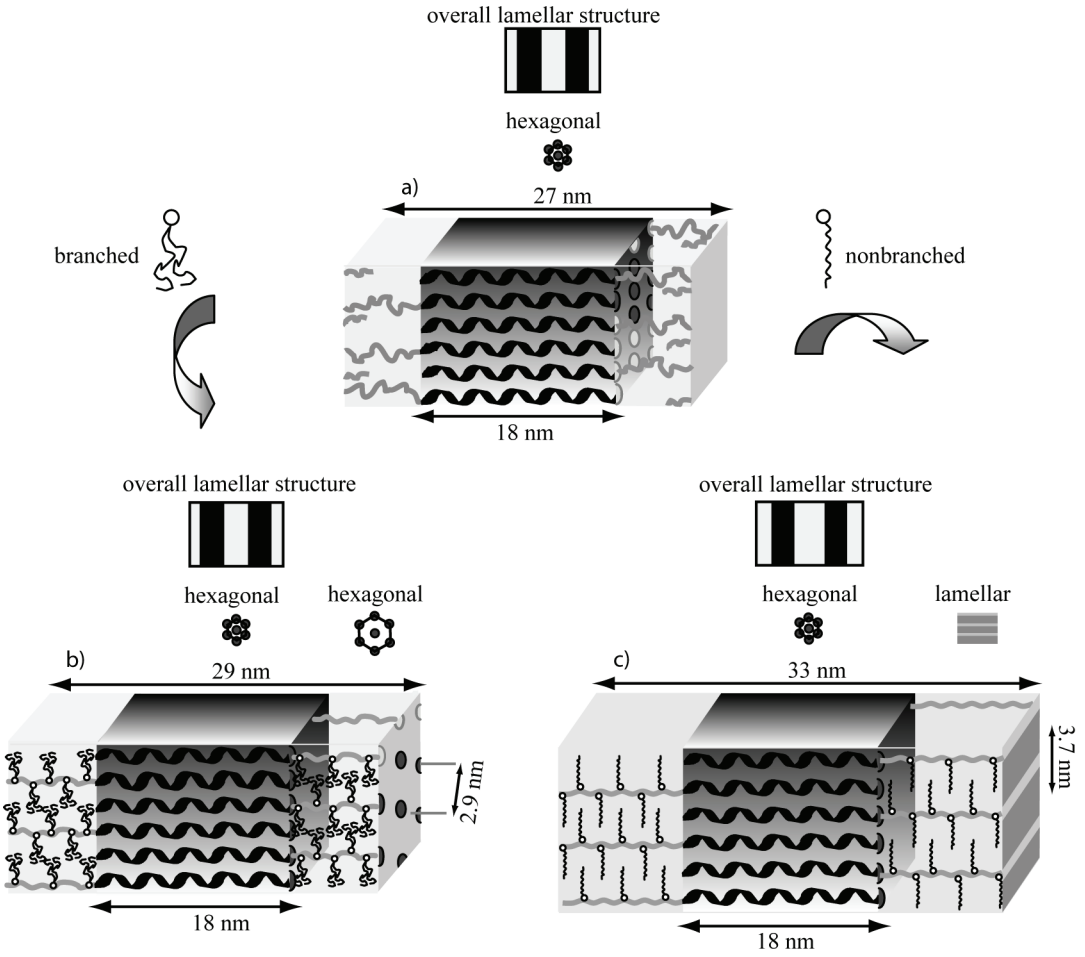

Figure 26. Proposed structures (strongly idealized). In all cases a lamellar overall order with alternating PBLG layers and PLys-containing layers is observed. The rod-like PBLG α-helices are hexagonally packed. a) In PBLG-*b*-PLys, the PLys domains have a disordered internal structure. b) In PBLG-*b*-PLys(DBSA)$_{1.0}$, a hexagonal assembly is suggested for PLys(DBSA)$_{1.0}$. c) In PBLG-*b*-PLys(DSA)$_{1.0}$, a lamellar self-assembly within the PLys(DSA)$_{1.0}$ phase is observed due to alternating α-helical PLys and DSA layers. Here the crystalline packing of *n*-dodecyl tails plays a role.

Figure 26 gives a strongly idealized picture on the self-assembly of the studied complexes. In reality, there must be an interfacial layer between the PBLG and PLys-surfactant domains, since adaptation between the structure sizes in these domains is needed. The packing of PBLG in the pure PBLG-*b*-PLys seems to be dominating for the lamellae formation, but as the order in the PLys-surfactant block increases, also PBLG domains have to adapt to the changes, which is shown by a slight broadening and shift of the PBLG packing reflection in SAXS. The surfactants studied in Article V are in the range of the size variation, to which the overall structure is able to adjust. However, in additional studies made on complexation of PBLG-*b*-PLys with the triple-tail lipid (diC16-PEG) described in the previous chapter, the large scale self-assembly was lost. The crystallization of the lipid alkyl tails became a dominant factor in self-assembly, inducing the oblique arrangement of PLys helices also in the diblock copolypeptide but inhibiting the PBLG packing due to the large size difference of the domains — thus, no block copolypeptide scale self-assembly was observed. We, however, were able to show the potential in controlling the secondary structures and self-assemblies in block copolypeptides, but also faced the fact that a careful selection of the construction units needs to be made in order to match the structural sizes of the different domains.

4 Conclusions

Polypeptides are an interesting group of polymers due to the relation of their constituent units to nature, where they serve as one of the most important building blocks for materials. From materials science point of view, they offer many attractive properties that are under study to work as building blocks or auxiliaries for new applications in synthetic materials.

In this thesis, the control of the secondary structures and self-assemblies of synthetically prepared homopolypeptides or block copolypeptides were studied in complexes with surfactant molecules. In Articles I and II, homopolypeptides PHis and PLys were complexed with a slightly branched single-tail surfactant DBSA leading to lamellar self-assembly. However, the secondary structures of the peptide backbones were found to be different, i.e. PPII-type helix in PHis and β-sheet in PLys, although the surfactant used for complexation was the same. This reveals the significance of different amino acids in designing new materials with different structures.

In Article III the amount of tails in the surfactant was increased to two. Three different phosphate surfactants, two with straight alkyl tails and one with branched alkyl tails, were complexed with PLys. Compared with the PLys complexed with the branched single-tail surfactant DBSA in Article II, the branching of the double-tail surfactant, i.e. diC2/6, induced changes in the secondary structure to α-helix as well as in the self-assembly of the complex. Instead of lamellar morphology, the complex assembled to a cylindrical phase with two coexisting local morphologies, hexagonal and tetragonal. The increased alkyl volume together with the branched architecture caused the transition. On the other hand, with the straight double alkyl tails, the changes were not that dramatic at room temperature. Despite the increased alkyl tail volume, the straight tails did not require much more lateral space compared with the DBSA complex, and thus a lamellar morphology was observed. However, the lamellar interlayer distance was increased.

The addition of still one more tail in the surfactant was shown in Article IV to lead to a material with structures at different length scales. Lipids with an asymmetric A_2B architecture having two alkyl tails and one hydrophilic PEG tail were complexed with three cationic homopolypeptides. The complexes self-assembled into a hierarchical layered structure with an oblique arrangement of the polypeptide helices. In comparison to the findings in Articles II and III, the bulky nature of the lipids was forcing PLys chains in α-helical conformation, which is typically observed to self-assemble in hexagonal morphology. This was indeed discovered with the lipid containing double bonds in the alkyl tails, being thus unable to crystallize. However, in case of the straight alkyl tails, crystallization forced the helices in a layered packing. The layered structure was transformed to a hexagonal packing of the helices upon heating the complexes above the melting temperature of the crystalline alkyl tails. The transition was found to be reversible. As the temperature was decreased back to room temperature, the oblique morphology returned.

Temperature behavior of the complexes was studied also in Articles I, II and III. The PLys complexes, studied in Articles II and III, showed changes in the morphology upon heating. The origin of the morphology transition, however, slightly differed from that found in Article IV. The transition from an oblique to a hexagonal morphology in Article IV took place due to the melting of the alkyl tail crystals, whereas in Article II the partial transition from hexagonal to lamellar was due to the refolding of the polypeptide backbone from α-helix to β-sheet. Furthermore, the partial transition from lamellar to cylindrical morphology in Article III can be described as a combination of the two previously mentioned mechanisms. At room temperature the alkyl tails in PLys(diC12)$_{1.0}$ were crystalline, but upon heating the melting alkyl tails required more space, thus inducing curvature of the peptide–surfactant interface and refolding of the peptide backbone from β-sheet to α-helix. These examples give a small glance on the underlying possibilities in selecting a proper surfactant for inducing desired functional properties.

In Articles II and III, also the processability of the polypeptide materials in the bulk state was taken under investigation. It was found that the addition of surfactant over the stoichiometric amount resulted in softening of the materials and thus improved processability properties. The additional surfactants were reasoned to interact through hydrogen bonds with the ionic complexes. Also the order of the self-assembly was found to enhance upon the addition of the surfactants.

Article V described the self-assembly of a diblock copolypeptide–surfactant complexes. The self-assembly was found to take place on three different length scales, where the diblock copolypeptide self-assembly formed the large scale lamellar structure, and both peptide blocks self-assembled independently on a smaller scale inside the lamellar phases. Hierarchical hexagonal-*and*-lamellar-*in*-lamellar and hexagonal-*and*-hexagonal-*in*-lamellar structures were reported.

The results of this thesis scratch the surface of the huge potential in polypeptide-based materials. It is no surprise that the field of nature-inspired materials has gained so much interest during the last couple of decades. Being natures own structural components, polypeptides are interesting also for the continuously growing field at the interface of biological and materials science. In conclusion, this thesis encourages pursuing novel rationally designed self-assemblies based on polypeptides to enable new schemes for biomimetic materials.

References

1. Camazine, S.; Deneubourg, J.-L.; Franks, N. R.; Theraulaz, G.; Bonabeau, E., *Self-Organization in Biological Systems*. Anderson, P. W.; Epstein, J. M.; Foley, D. K.; Levin, S. A.; Nowak, M. A., Eds. Princeton University Press: Oxfordshire, 2001, p. 538.
2. Whitesides, G. M.; Mathias, J. P.; Seto, C. T. *Molecular Self-Assembly and Nanochemistry: A Chemical Strategy for the Synthesis of Nanostructures.* Science **1991,** 254, 1312-1319.
3. Muthukumar, M.; Ober, C. K.; Thomas, E. L. *Competing Interactions and Levels of Ordering in Self-Organizing Polymeric Materials.* Science **1997,** 277, 1225-1232.
4. Bates, F. S.; Fredrickson, G. H. *Block Copolymer Thermodynamics: Theory and Experiments.* Annu. Rev. Phys. Chem. **1990,** 41, 525-557.
5. Hamley, I. W., *The Physics of Block Copolymers*. Oxford University Press, Oxford, 1998, p. 424.
6. Bates, F. S.; Fredrickson, G. H. *Block Copolymers - Designer Soft Materials.* Phys. Today **1999,** 52 (2) 32-38.
7. Zheng, W.; Wang, Z.-G. *Morphology of ABC Triblock Copolymers.* Macromolecules **1995,** 28 (21) 7215-7223.
8. Fischer, H.; Poser, S. *Liquid Crystalline Block and Graft Copolymers.* Acta Polymer **1996,** 47 (10) 413-428.
9. Hadjichristidis, N.; Pitsikalis, M.; Pispas, S.; Iatrou, H. *Polymers with Complex Architecture by Living Anionic Polymerization.* Chem. Rev. **2001,** 101 (12) 3747-3792.
10. Karatzas, A.; Iatrou, H.; Hadjichristidis, N.; Inoue, K.; Sugiyama, K.; Hirao, A. *Complex Macromolecular Chimeras.* Biomacromolecules **2008,** 9 (7) 2072-2080.
11. Abetz, V.; Goldacker, T. *Formation of Superlattices via Blending of Block Copolymers.* Macromol. Rapid Commun. **2000,** 21, 16-34.
12. Klok, H.-A.; Lecommandoux, S. *Supramolecular Materials via Block Copolymer Self-Assembly.* Adv. Mater. **2001,** 13 (16) 1217-1229.
13. Klok, H.-A.; Langenwalter, J. F.; Lecommandoux, S. *Self-Assembly of Peptide-Based Diblock Oligomers.* Macromolecules **2000,** 33 (21) 7819-7826.
14. Radzilowski, L. H.; Carragher, B. O.; Stupp, S. I. *Three-Dimensional Self-Assembly of Rodcoil Copolymer Nanostructures.* Macromolecules **1997,** 30 (7) 2110-2119.
15. Lee, M.; Cho, B.-K.; Zin, W.-C. *Supramolecular Structures from Rod-Coil Block Copolymers.* Chem. Rev. **2001,** 101 (12) 3869-3892.
16. Chen, J. T.; Thomas, E. L.; Ober, C. K.; Mao, G.-p. *Self-Assembled Smectic Phases in Rod-Coil Block Copolymers.* Science **1996,** 273 (5273) 343-346.
17. Olsen, B. D.; Segalman, R. A. *Self-Assembly of Rod-Coil Block Copolymers.* Mater. Sci. Eng., R **2008,** 62 (2) 37-66.
18. Stupp, S. I.; LeBonheur, V.; Walker, K.; Li, L. S.; Huggins, K. E.; Keser, M.; Amstutz, A. *Supramolecular Materials: Self-Organized Nanostructures.* Science **1997,** 276 (5311) 384-389.
19. Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. *DNA-Templated Self-Assembly of Protein Arrays and Highly Conductive Nanowires.* Science **2003,** 301 (5641) 1882-1884.
20. Jaeger, L.; Chworos, A. *The Architectonics of Programmable RNA and DNA Nanostructures.* Curr. Opin. Struct. Biol. **2006,** 16 (4) 531-543.
21. Feldkamp, U.; Niemeyer, C. M. *Rational Design of DNA Nanoarchitectures.* Angew. Chem. Int. Ed. **2006,** 45, 1856-1876.
22. Seeman, N. C. *DNA in a Material World.* Nature **2003,** 421 (6921) 427-431.
23. Alemdaroglu, F. E.; Herrmann, A. *DNA Meets Synthetic Polymers-Highly Versatile Hybrid Materials.* Org. Biomol. Chem. **2007,** 5 (9) 1311-1320.
24. Douglas, T.; Young, M. *Viruses: Making Friends with Old Foes.* Science **2006,** 312 (5775) 873-875.
25. Lee, L.; Niu, Z.; Wang, Q. *Viruses and Virus-like Protein Assemblies—Chemically Programmable Nanoscale Building Blocks.* Nano Res. **2009,** 2 (5) 349-364.
26. Kostiainen, M. A.; Kasyutich, O.; Cornelissen, J. J. L. M.; Nolte, R. J. M. *Self-Assembly and Optically Triggered Disassembly of Hierarchical Dendron-Virus Complexes.* Nature Chem. **2010,** 2 (5) 394-399.
27. Branden, C.; Tooze, J., *Introduction to Protein Structure*. 2nd ed.; Garland Publishing, New York, 1999, p. 410.
28. Vandermeulen, G. W. M.; Tziatzios, C.; Klok, H.-A. *Reversible Self-Organization of Poly(ethylene glycol)-Based Hybrid Block Copolymers Mediated by a De Novo Four-Stranded α-Helical Coiled Coil Motif.* Macromolecules **2003,** 36 (11) 4107-4114.
29. Hamley, I. *Peptide Fibrillization.* Angew. Chem. Int. Ed. **2007,** 46, 8128-8147.
30. Vandermeulen, G. W. M.; Klok, H.-A. *Peptide/Protein Hybrid Materials: Enhanced Control of Structure and Improved Performance through Conjugation of Biological and Synthetic Polymers.* Macromol. Biosci. **2004,** 4 (4) 383-398.
31. Börner, H. G.; Schlaad, H. *Bioinspired Functional Block Copolymers.* Soft Matter **2007,** 3 (4) 394-408.

32. Knowles, T. P. J.; Oppenheim, T. W.; Buell, A. K.; Chirgadze, D. Y.; Welland, M. E. *Nanostructured Films from Hierarchical Self-Assembly of Amyloidogenic Proteins.* Nature Nanotech. **2010,** 5 (3) 204-207.
33. Gallot, B.; Fafiotte, M.; Fissi, A.; Pieroni, O. *Liquid-Crystalline Structure of Poly(L-lysine) Containing Azobenzene Units in the Side Chain.* Macromol. Rapid Commun. **1996,** 17 (8) 493-501.
34. Ponomarenko, E. A.; Waddon, A. J.; Bakeev, K. N.; Tirrell, D. A.; MacKnight, W. J. *Self-Assembled Complexes of Synthetic Polypeptides and Oppositely Charged Low Molecular Weight Surfactants. Solid-State Properties.* Macromolecules **1996,** 29, 4340-4345.
35. Lecommandoux, S.; Achard, M.-F.; Langenwalter, J. F.; Klok, H.-A. *Self-Assebly of Rod-Coil Diblock Oligomers Based on α-Helical Peptides.* Macromolecules **2001,** 34, 9100-9111.
36. Houbenov, N.; Nykänen, A.; Iatrou, H.; Hadjichristidis, N.; Ruokolainen, J.; Faul, C. F. J.; Ikkala, O. *Fibrillar Constructs from Multilevel Hierarchical Self-Assembly of Discotic and Calamitic Supramolecular Motifs.* Adv. Funct. Mater. **2008,** 18 (14) 2041-2047.
37. Hanski, S.; Houbenov, N.; Ruokolainen, J.; Chondronicola, D.; Iatrou, H.; Hadjichristidis, N.; Ikkala, O. *Hierarchical Ionic Self-Assembly of Rod-Comb Block Copolypeptide-Surfactant Complexes.* Biomacromolecules **2006,** 7, 3379-3384.
38. Hanski, S.; Junnila, S.; Almasy, L.; Ruokolainen, J.; Ikkala, O. *Structural and Conformational Transformations in Self-Assembled Polypeptide-Surfactant Complexes.* Macromolecules **2008,** 41 (3) 866-872.
39. Junnila, S.; Hanski, S.; Oakley, R. J.; Nummelin, S.; Ruokolainen, J.; Faul, C. F.; Ikkala, O. *Effect of Double-Tailed Surfactant Architecture on the Conformation, Self-Assembly, and Processing in Polypeptide-Surfactant Complexes.* Biomacromolecules **2009,** 10 (10) 2787-2794.
40. Binder, W.; Bernstorff, S.; Kluger, C.; Petraru, L.; Kunz, M. *Tunable Materials from Hydrogen-Bonded Pseudo Block Copolymers.* Adv. Mater. **2005,** 17 (23) 2824-2828.
41. Noro, A.; Nagata, Y.; Takano, A.; Matsushita, Y. *Diblock-Type Supramacromolecule via Biocomplementary Hydrogen Bonding.* Biomacromolecules **2006,** 7 (6) 1696-1699.
42. Brunsveld, L.; Folmer, B. J. B.; Meijer, E. W.; Sijbesma, R. P. *Supramolecular Polymers.* Chem. Rev. **2001,** 101 (12) 4071-4097.
43. Faul, C. F. J.; Antonietti, M. *Ionic Self-Assembly: Facile Synthesis of Supramolecular Materials.* Adv. Mater. **2003,** 15 (9) 673-683.
44. Ober, K.; Wegner, G. *Polyelectrolyte-Surfactant Complexes in the Solid State: Facile Building Blocks for Self-Organizing Materials.* Adv. Mater. **1997,** 9 (1) 17-31.
45. Antonietti, M.; Conrad, J.; Thünemann, A. *Polyelectrolyte-Surfactant Complexes: A New Type of Solid, Mesomorphous Material.* Macromolecules **1994,** 27, 6007-6011.
46. Antonietti, M.; Burger, C.; Thünemann, A. *Polyelectrolyte-Surfactant Complexes: A New Class of Highly Ordered Polymer Materials.* TRIP **1997,** 5 (8) 262-267.
47. Ikkala, O.; Ruokolainen, J.; ten Brinke, G.; Torkkeli, M.; Serimaa, R. *Mesomorphic State of Poly(vinylpyridine)-Dodecylbenzenesulfonic Acid Complexes in Bulk and in Xylene Solution.* Macromolecules **1995,** 28, 7088-7094.
48. Zhou, S.; Chu, B. *Assembled Materials: Polyelectrolyte–Surfactant Complexes.* Adv. Mater. **2000,** 12 (8) 545-556.
49. Chen, H.-L.; Hsiao, M.-S. *Self-Assembled Mesomorphic Complexes of Branched Poly(ethylenimine) and Dodecylbenzenesulfonic Acid.* Macromolecules **1999,** 32 (9) 2967-2973.
50. Levon, K.; Ho, K. H.; Zheng, W. Y.; Laakso, J.; Kärnä, T.; Taka, T.; Österholm, J. E. *Thermal Doping of Polyaniline with Dodecylbenzene Sulfonic Acid without Auxiliary Solvents.* Polymer **1995,** 36 (14) 2733-2738.
51. Dufour, B.; Rannou, P.; Fedorko, P.; Djurado, D.; Travers, J.-P.; Pron, A. *Effect of Plasticizing Dopants on Spectroscopic Properties, Supramolecular Structure, and Electrical Transport in Metallic Polyaniline.* Chem. Mater. **2001,** 13 (11) 4032-4040.
52. Belfiore, L. A.; Pires, A. T. N.; Wang, Y.; Graham, H.; Ueda, E. *Transition-metal Coordination in Polymer Blends and Model Systems.* Macromolecules **1992,** 25 (5) 1411-1419.
53. Ruokolainen, J.; Tanner, J.; ten Brinke, G.; Ikkala, O.; Torkkeli, M.; Serimaa, R. *Poly(4-vinyl pyridine)/Zinc Dodecyl Benzene Sulfonate Mesomorphic State Due to Coordination Complexation.* Macromolecules **1995,** 28 (23) 7779-7784.
54. Valkama, S.; Ruotsalainen, T.; Kosonen, H.; Ruokolainen, J.; Torkkeli, M.; Serimaa, R.; ten Brinke, G.; Ikkala, O. *Amphiphiles Coordinated to Block Copolymers as a Template for Mesoporous Materials.* Macromolecules **2003,** 36 (11) 3986-3991.
55. Valkama, S.; Lehtonen, O.; Lappalainen, K.; Kosonen, H.; Castro, P.; Repo, T.; Torkkeli, M.; Serimaa, R.; ten Brinke, G.; Leskelä, M.; Ikkala, O. *Multicomb Polymeric Supramolecules and Their Self-Organization: Combination of Coordination and Ionic Interactions.* Macromol. Rapid Commun. **2003,** 24 (9) 556-560.
56. Kurth, D.; Fromm, K.; Lehn, J. M. *Hydrogen-Bonding and Metal-ion-mediated Self-Assembly of a Nanoporous Crystal Lattice.* Eur. J. Inorg. Chem. **2001,** 2001, 1523-1526.
57. Ikkala, O.; ten Brinke, G. *Functional Materials Based on Self-Assembly of Polymeric Supramolecules.* Science **2002,** 295, 2407-2409.
58. Ruokolainen, J.; Mäkinen, R.; Torkkeli, M.; Mäkelä, T.; Serimaa, R.; ten Brinke, G.; Ikkala, O. *Switching Supramolecular Polymeric Materials with Multiple Length Scales.* Science **1998,** 280 (5363) 557-560.

59. Kato, T.; Mizoshita, N.; Kishimoto, K. *Functional Liquid-Crystalline Assemblies: Self-Organized Soft Materials.* Angew. Chem. Int. Ed. Engl. **2006,** 45, 38-68.
60. Tal'roze, R. V.; Kuptsov, S. A.; Sycheva, T. I.; Bezborodov, V. S.; Plate, N. A. *Order and Liquid Crystalline Phase Behavior of Polyacid-Tertiary Amine Complexes.* Macromolecules **1995,** 28 (25) 8689-8691.
61. Antonietti, M.; Henke, S.; Thünemann, A. *Highly Ordered Materials with Ultra-low Surface Energies: Polyelectrolyte–Surfactant Complexes with Fluorinated Surfactants.* Adv. Mater. **1996,** 8 (1) 41-45.
62. Ruokolainen, J.; Tanner, J.; Ikkala, O.; ten Brinke, G.; Thomas, E. L. *Direct Imaging of Self-Organized Comb Copolymer-like Systems Obtained by Hydrogen Bonding: Poly(4-vinylpyridine)-4-nonadecylphenol.* Macromolecules **1998,** 31 (11) 3532-3536.
63. Antonietti, M.; Wenzel, A.; Thünemann, A. *The "Egg-Carton" Phase: A New Morphology of Complexes of Polyelectrolytes with Natural Lipid Mixtures.* Langmuir **1996,** 12 (8) 2111-2114.
64. Antonietti, M.; Maskos, M. *Fine-Tuning of Phase Structures and Thermoplasticity of Polyelectrolyte-Surfactant Complexes: Copolymers of Ionic Monomers with N-Alkylacrylamides.* Macromolecules **1996,** 29, 4199-4205.
65. Hayakawa, K.; Kwak, J. C. T. *Surfactant-Polyelectrolyte Interactions. 1. Binding of Dodecyltrimethylammonium Ions by Sodium Dextransulfate and Sodium Poly(styrenesulfonate) in Aqueous Solution in the Presence of Sodium Chloride.* J. Phys. Chem. **1982,** 86 (19) 3866-3870.
66. Hayakawa, K.; Kwak, J. C. T. *Study of Surfactant-Polyelectrolyte Interactions. 2. Effect of Multivalent Counterions on the Binding of Dodecyltrimethylammonium Ions by Sodium Dextran Sulfate and Sodium Poly(styrene sulfonate) in Aqueous Solution.* J. Phys. Chem. **1983,** 87 (3) 506-509.
67. Antonietti, M.; Conrad, J. *Synthesis of Very Highly Ordered Liquid Crystalline Phases by Complex Formation of Polyacrylic Acid with Cationic Surfactants.* Angew. Chem. Int. Ed. **1994,** 33 (18) 1869-1870.
68. Ponomarenko, E. A.; Tirrell, D. A.; MacKnight, W. J. *Stoichiometric Complexes of Synthetic Polypeptides and Oppositely Charged Surfactants in Organic Solvents and in the Solid State.* Macromolecules **1996,** 29 (27) 8751-8758.
69. Wenzel, A.; Antonietti, M. *Superstructures of Lipid Bilayers by Complexation with Helical Biopolymers.* Adv. Mater. **1997,** 9, 487-490.
70. Ponomarenko, E. A.; Waddon, A. J.; Bakeev, K. N.; Tirrel, D. A.; MacKnight, W. J. *Self-Assembled Complexes of Synthetic Polypeptides and Oppositely Charged Low Molecular Weight Surfactants. Solid-State Porperties.* Macromolecules **1996,** 29, 4340-4345.
71. Canilho, N.; Scholl, M.; Klok, H.-A.; Mezzenga, R. *Thermotropic Ionic Liquid Crystals via Self-Assembly of Cationic Hyperbranched Polypeptides and Anionic Surfactants.* Macromolecules **2007,** 40 (23) 8374-8383.
72. Schaefer, K. E.; Keller, P.; Deming, T. J. *Thermotropic Polypeptides Bearing Side-On Mesogens.* Macromolecules **2006,** 39 (1) 19-22.
73. Gallot, B.; Fafiotte, M.; Fissi, A.; Pieroni, O. *Poly(L-lysine) Containing Azobenzene Units in the Side Chains: Influence of the Degree of Substitution on Liquid Crystalline Structure and Thermotropic Behaviour.* Liq. Cryst. **1997,** 23 (1) 137-146.
74. Watanabe, J.; Fukuda, Y.; Gehani, R.; Uematsu, I. *Thermotropic Polypeptides. 1. Investigation of Cholesteric Mesophase Properties of Poly(γ-methyl-D-glutamate-co-γ-hexyl-D-glutamate)s.* Macromolecules **1984,** 17 (5) 1004-1009.
75. Watanabe, J.; Ono, H.; Uematsu, I.; Abe, A. *Thermotropic Polypeptides. 2. Molecular Packing and Thermotropic Behavior of Poly(L-glutamates) with Long n-alkyl Side Chains.* Macromolecules **1985,** 18 (11) 2141-2148.
76. Watanabe, J.; Takashina, Y. *Columnar Liquid Crystals in Polypeptides. 1. A Columnar Hexagonal Liquid Crystal Observed in Poly(γ-octadecyl-L-glutamate).* Macromolecules **1991,** 24 (11) 3423-3426.
77. Watanabe, J.; Tominaga, T. *Thermotropic Liquid Crystals in Polypeptides with Mesogenic Side Chains. 1.* Macromolecules **1993,** 26 (15) 4032-4036.
78. Zheng, W.-Y.; Wang, R.-H.; Levon, K.; Rong, Z. Y.; Taka, T.; Pan, W. *Self-Assembly of the Electroactive Complexes of Polyaniline and Surfactant.* Macromol. Chem. Phys. **1995,** 196 (8) 2443-2462.
79. Cao, Y.; Smith, P.; Heeger, A. J. *Counter-ion Induced Processibility of Conducting Polyaniline and of Conducting Polyblends of Polyaniline in Bulk Polymers.* Synth. Met. **1992,** 48 (1) 91-97.
80. Kosonen, H.; Valkama, S.; Ruokolainen, J.; Torkkeli, M.; Serimaa, R.; ten Brinke, G.; Ikkala, O. *One-Dimensional Optical Reflectors Based on Self-Organization of Polymeric Comb-Shaped Supramolecules.* Eur. Phys. J. E **2003,** 10 (1) 69-75.
81. Knaapila, M.; Ruokolainen, J.; Torkkeli, M.; Serimaa, R.; Horsburgh, L.; Monkman, A. P.; Bras, W.; ten Brinke, G.; Ikkala, O. *Self-Organized Supramolecules of Poly(2,5-pyridinediyl).* Synth. Met. **2001,** 121 (1-3) 1257-1258.
82. Adzhubei, A. A.; Sternberg, M. J. E. *Left-handed Polyproline II Helices Commonly Occur in Globular Proteins.* J. Mol. Biol. **1993,** 229, 472-493.
83. Lefèvre, T.; Leclerc, J.; Rioux-Dube, J.-F.; Buffeteau, T.; Paquin, M.-C.; Rousseau, M.-E.; Cloutier, I.; Auger, M.; Gagné, S. M.; Boudreault, S.; Cloutier, C.; Pézolet, M. *Conformation of Spider Silk Proteins In Situ in the Intact Major Ampullate Gland and in Solution.* Biomacromolecules **2007,** 8, 2342-2344.
84. Chellgren, B. W.; Creamer, T. P. *Short Sequences of Non-Proline Residues can Adopt the Polyproline II Helical Conformation.* Biochemistry **2004,** 43, 5864-5869.

85. Ponomarenko, E. A.; Tirrel, D. A.; MacKnight, W. J. *Water-Insoluble Complexes of Poly(L-Lysine) with Mixed Alkyl Sulfates: Composition-Controlled Solid State Structures.* Macromolecules **1998,** 31, 1584-1589.
86. Vikki, T.; Ikkala, O. T. *On the Dynamic-Mechanical Relaxations of Polyaniline (Dodecyl benzene sulphonic acid) - Salt.* Synth. Met. **1995,** 69 (1-3) 235-236.
87. Ikkala, O. T.; Pietilä, L. O.; Passiniemi, P.; Vikki, T.; Österholm, H.; Ahjopalo, L.; Österholm, J. E. *Processible Polyaniline Complexes Due to Molecular Recognition: Supramolecular Structures Based on Hydrogen Bonding and Phenyl Stacking.* Synth. Met. **1997,** 84 (1-3) 55-58.
88. Pron, A.; Rannou, P. *Processible Conjugated Polymers: from Organic Semiconductors to Organic Metals and Superconductors.* Prog. Polym. Sci. **2002,** 27 (1) 135-190.
89. Dufour, B.; Rannou, P.; Djurado, D.; Zagorska, M.; Kulszewicz-Bajer, I.; Pron, A. *The Role of Chain and Dopant Engineering in the Preparation of Processible Conducting Polymers with Desired Properties.* Synth. Met. **2003,** 135-136, 63-68.
90. Olinga, T. E.; Fraysse, J.; Travers, J. P.; Dufresne, A.; Pron, A. *Highly Conducting and Solution-Processable Polyaniline Obtained via Protonation with a New Sulfonic Acid Containing Plasticizing Functional Groups*. Macromolecules **2000,** 33 (6) 2107-2113.
91. Valkama, S.; Ruotsalainen, T.; Nykänen, A.; Laiho, A.; Kosonen, H.; ten Brinke, G.; Ikkala, O.; Ruokolainen, J. *Self-Assembled Structures in Diblock Copolymers with Hydrogen-Bonded Amphiphilic Plasticizing Compounds.* Macromolecules **2006,** 39 (26) 9327-9336.
92. Kosonen, H.; Valkama, S.; Ruokolainen, J.; Ten Brinke, G.; Ikkala, O. *Polymeric One-Dimensional Reflectors Based on Self-Organzation of Comb-Shaped Supramolecules.* Mat. Res. Soc. Symp. Proc. **2003,** 775, P6.18.1-P6.18.6.
93. Kosonen, H.; Ruokolainen, J.; Knaapila, M.; Torkkeli, M.; Jokela, K.; Serimaa, R.; ten Brinke, G.; Bras, W.; Monkman, A. P.; Ikkala, O. *Nanoscale Conducting Cylinders Based on Self-Organization of Hydrogen-Bonded Polyaniline Supramolecules.* Macromolecules **2000,** 33 (23) 8671-8675.
94. Ikkala, O.; Knaapila, M.; Ruokolainen, J.; Torkkeli, M.; Serimaa, R.; Jokela, K.; Horsburgh, L.; Monkman, A.; Brinke, G. t. *Self-Organized Liquid Phase and Co-Crystallization of Rod-Like Polymers Hydrogen-Bonded to Amphiphilic Molecules.* Adv. Mater. **1999,** 11 (14) 1206-1210.
95. Knaapila, M.; Stepanyan, R.; Horsburgh, L. E.; Monkman, A. P.; Serimaa, R.; Ikkala, O.; Subbotin, A.; Torkkeli, M.; ten Brinke, G. *Structure and Phase Equilibria of Polyelectrolytic Hairy-Rod Supramolecules in the Melt State.* J. Phys. Chem. B **2003,** 107 (51) 14199-14203.
96. Simmons, A. H.; Michal, C. A.; Jelinski, L. W. *Molecular Orientation and Two-Component Nature of the Crystalline Fraction of Spider Dragline Silk.* Science **1996,** 271 (5245) 84-87.
97. Hornyak, G. L.; Dutta, J.; Tibbals, H. F.; Rao, A. K., *Introduction to Nanoscience*. Taylor & Francis Group, Boca Raton, 2008, p. 815.
98. O'Brien, J. P.; Fahnestock, S. R.; Termonia, Y.; Gardner, K. H. *Nylons from Nature: Synthetic Analogs to Spider Silk.* Adv. Mater. **1998,** 10 (15) 1185-1195.
99. Hardy, J. G.; Scheibel, T. R. *Silk-Inspired Polymers and Proteins.* Biochem. Soc. Trans. **2009,** 37, 677-681.
100. Smeenk, J. M.; Schön, P.; Otten, M. B. J.; Speller, S.; Stunnenberg, H. G.; van Hest, J. C. M. *Fibril Formation by Triblock Copolymers of Silklike β-Sheet Polypeptides and Poly(ethylene glycol).* Macromolecules **2006,** 39 (8) 2989-2997.
101. Rathore, O.; Sogah, D. Y. *Self-Assembly of β-Sheets into Nanostructures by Poly(alanine) Segments Incorporated in Multiblock Copolymers Inspired by Spider Silk.* J. Am. Chem. Soc. **2001,** 123 (22) 5231-5239.
102. Smeenk, J. M.; Otten, M. B. J.; Thies, J.; Tirrell, D. A.; Stunnenberg, H. G.; van Hest, J. C. M. *Controlled Assembly of Macromolecular β-Sheet Fibrils.* Angew. Chem. Int. Ed. **2005,** 44 (13) 1968-1971.
103. Hardy, J. G.; Römer, L. M.; Scheibel, T. R. *Polymeric Materials Based on Silk Proteins.* Polymer **2008,** 49 (20) 4309-4327.
104. Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Pispas, S.; Avgeropoulos, A. *Linear and Non-Linear Triblock Terpolymers. Synthesis, Self-Assembly in Selective Solvents and in Bulk.* Prog. Polym. Sci. **2005,** 30, 725-782.
105. Masuda, J.; Takano, A.; Nagata, Y.; Noro, A.; Matsushita, Y. *Nanophase-Separated Synchronizing Structure with Parallel Double Periodicity from an Undecablock Terpolymer.* Phys. Rev. Lett. **2006,** 97 (9) 098301.
106. Auschra, C.; Stadler, R. *New Ordered Morphologies in ABC Triblock Copolymers.* Macromolecules **1993,** 26 (9) 2171-2174.
107. Jenekhe, S. A.; Chen, X. L. *Self-Assembly of Ordered Microporous Materials from Rod-Coil Block Copolymers.* Science **1999,** 283 (5400) 372-375.
108. Ikkala, O.; ten Brinke, G. *Functional Materials Based on Self-Assembly of Polymeric Supramolecules.* Science **2002,** 295, 2407-2409.
109. Ruokolainen, J.; ten Brinke, G.; Ikkala, O. *Supramolecular Polymeric Materials with Hierarchical Structure-within-Structure Morphologies.* Adv. Mater. **1999,** 11 (9) 777-780.
110. Wang, L.; Lin, J.; Zhang, L. *Hierarchically Ordered Microstructures Self-Assembled from Comb-Coil Block Copolymers.* Langmuir **2009,** 25 (8) 4735-4742.
111. Thünemann, A. F.; General, S. *Poly(ethylene oxide)-b-poly(ethylene imine) Dodecanoate Complexes: Lamellar-within-Lamellar Morphologies and Nanoparticles.* Macromolecules **2001,** 34 (20) 6978-6984.

112. Ikkala, O.; Ras, R. H. A.; Houbenov, N.; Ruokolainen, J.; Pääkkö, M.; Laine, J.; Leskela, M.; Berglund, L. A.; Lindstrom, T.; ten Brinke, G.; Iatrou, H.; Hadjichristidis, N.; Faul, C. F. J. *Solid State Nanofibers Based on Self-Assemblies: from Cleaving from Self-Assemblies to Multilevel Hierarchical Constructs.* Faraday Discuss. **2009,** 143, 95-107.
113. Schlaad, H.; Smarsly, B.; Losik, M. *The Role of Chain-Length Distribution in the Formation of Solid-State Structures of Polypeptide-Based Rod-Coil Block Copolymers.* Macromolecules **2004,** 37, 2210-2214.
114. Schlaad, H.; Kukula, H.; Smarsly, B.; Antonietti, M.; Pakula, T. *Solid-State Morphologies of Linear and Bottlebrush-Shaped Polystyrene-poly(Z-L-lysine) Block Copolymers.* Polymer **2002,** 43, 5321-5328.
115. Losik, M.; Kubowicz, S.; Smarsly, B.; Schlaad, H. *Solid-State Structure of Polypeptide-Based Rod-Coil Block Copolymers: Folding of Helices.* Eur. Phys. J. E **2004,** 15 (4) 407-411.
116. Gitsas, A.; Floudas, G.; Mondeshki, M.; Butt, H. J.; Spiess, H. W.; Iatrou, H.; Hadjichristidis, N. *Effect of Chain Topology on the Self-Organization and Dynamics of Block Copolypeptides: From Diblock Copolymers to Stars.* Biomacromolecules **2008,** 9 (7) 1959-1966.
117. Gitsas, A.; Floudas, G.; Mondeshki, M.; Lieberwirth, I.; Spiess, H. W.; Iatrou, H.; Hadjichristidis, N.; Hirao, A. *Hierarchical Self-Assembly and Dynamics of a Miktoarm Star Chimera Composed of Poly(γ-benzyl-L-glutamate), Polystyrene, and Polyisoprene.* Macromolecules **2010,** 43 (4) 1874-1881.
118. Babin, J.; Taton, D.; Brinkmann, M.; Lecommandoux, S. *Synthesis and Self-Assembly in Bulk of Linear and Mikto-Arm Star Block Copolymers Based on Polystyrene and Poly(glutamic acid).* Macromolecules **2008,** 41 (4) 1384-1392.
119. Hammond, M. R.; Klok, H. A.; Mezzenga, R. *Self-Organization on Multiple Length Scales in "Hairy Rod"-Coil Block Copolymer Supramolecular Complexes.* Macromol. Rapid Commun. **2008,** 29, 299-303.
120. Thünemann, A.; Beyermann, J.; Kukula, H. *Poly(ethylene oxide)-b-poly(L-lysine) Complexes with Retinoic Acid.* Macromolecules **2000,** 33 (16) 5906-5911.
121. Barenholz, Y. *Liposome Application: Problems and Prospects.* Curr. Opin. Coll. Int. Sci. **2001,** 6 (1) 66-77.
122. Matsushita, Y.; Takano, A.; Hayashida, K.; Asari, T.; Noro, A. *Hierarchical Nanophase-Separated Structures Created by Precisely-Designed Polymers with Complexity.* Polymer **2009,** 50 (10) 2191-2203.
123. Soininen, A. J.; Kasëmi, E.; Schlüter, A. D.; Ikkala, O.; Ruokolainen, J.; Mezzenga, R. *Self-Assembly and Induced Circular Dichroism in Dendritic Supramolecules with Cholesteric Pendant Groups.* J. Am. Chem. Soc. **2010,** 132 (31) 10882-10890.
124. Zhou, S.; Hu, H.; Burger, C.; Chu, B. *Phase Structural Transitions of Polyelectrolyte-Surfactant Complexes between Poly(vinylamine hydrochloride) and Oppositely Charged Sodium Alkyl Sulfate.* Macromolecules **2001,** 34 (6) 1772-1778.
125. Artzner, F.; Zantl, R.; Rapp, G.; Rädler, J. O. *Observation of a Rectangular Columnar Phase in Condensed Lamellar Cationic Lipid-DNA Complexes.* Phys. Rev. Lett. **1998,** 81 (22) 5015-5018.
126. Koltover, I.; Salditt, T.; Safinya, C. R. *Phase Diagram, Stability, and Overcharging of Lamellar Cationic Lipid DNA Self-Assembled Complexes.* Biophys. J. **1999,** 77 (2) 915-924.
127. McManus, J. J.; Rädler, J. O.; Dawson, K. A. *Observation of a Rectangular Columnar Phase in a DNA-Calcium-Zwitterionic Lipid Complex.* J. Am. Chem. Soc. **2004,** 126 (49) 15966-15967.
128. Koltover, I.; Sahu, S.; Davis, N. *Genetic Engineering of the Nanoscale Structure in Polyelectrolyte-Lipid Self-Assembled Systems.* Angew. Chem. Int. Ed. **2004,** 43 (31) 4034-4037.
129. Flory, P. J.; Vrij, A. *Melting Points of Linear-Chain Homologs. The Normal Parraffin Hydrocarbons.* J. Am. Chem. Soc. **1963,** 85, 3548-3553.
130. Jordan, E. F.; Artymyshyn, B.; Speca, A.; Wrigley, A. N. *Side-Chain Crystallinity. II. Heats of Fusion and Melting Transitions on Selected Copolymers Incorporating n-Octadecyl Acrylate or Vinyl Stearate.* J. Polym. Sci. Part A1 **1971,** 9 (11) 3349-3365.
131. Deming, T. J. *Facile Synthesis of Block Copolypeptides of Defined Architecture.* Nature **1997,** 390 (6658) 386-389.
132. Deming, T. J. *Living Polymerization of α-amino acid-N-carboxyanhydrides.* J. Polym. Sci., Part A: Polym. Chem. **2000,** 38 (17) 3011-3018.
133. Dimitrov, I.; Kukula, H.; Cölfen, H.; Schlaad, H. *Advances in the Synthesis and Characterization of Polypeptide-Based Hybrid Block Copolymers.* Macromol. Symp. **2004,** 215, (Proceedings of the 2003 International Symposium on Ionic Polymerization and Related Processes), 383-393.
134. Aliferis, T.; Iatrou, H.; Hadjichristidis, N. *Living Polypeptides.* Biomacromolecules **2004,** 5 (5) 1653-1656.
135. Schlaad, H.; Smarsly, B.; Below, I. *Solid-State Structure of Polystyrene-block-poly(γ-benzyl-L-glutamate): Helix Folding vs Stretching.* Macromolecules **2006,** 39 (13) 4631-4632.
136. Ludwigs, S.; Krausch, G.; Reiter, G.; Losik, M.; Antonietti, M.; Schlaad, H. *Structure Formation of Polystyrene-block-poly(γ-benzyl-L-glutamate) in Thin Films.* Macromolecules **2005,** 38 (18) 7532-7535.
137. Papadopoulos, P.; Floudas, G.; Schnell, I.; Aliferis, T.; Iatrou, H.; Hadjichristidis, N. *Nanodomain-Induced Chain Folding in Poly(γ-benzyl-L-glutamate)-b-polyglycine Diblock Copolymers.* Biomacromolecules **2005,** 6 (4) 2352-2361.
138. Minich, E. A.; Nowak, A. P.; Deming, T. J.; Pochan, D. J. *Rod-Rod and Rod-Coil Self-Assembly and Phase Behavior of Polypeptide Diblock Copolymers.* Polymer **2004,** 45 (6) 1951-1957.
139. Floudas, G.; Papadopoulos, P.; Klok, H.-A.; Vandermeulen, G. W. M.; Rodriguez-Hernandez, J. *Hierarchical Self-Assembly of Poly(γ-benzyl-L-glutamate)-Poly(ethylene glycol)-Poly(γ-benzyl-L-glutamate) Rod-Coil-Rod Triblock Copolymers.* Macromolecules **2003,** 36 (10) 3673-3683.

Abstracts of Publications I-V

I We present lamellar self-assembly of cationic poly(L-histidine) (PLH) stoichiometrically complexed with an anionic surfactant, dodecyl benzenesulfonic acid (DBSA), which allows a stabilized conformation reminiscent of polyproline type II (PPII) left-handed helices. Such a conformation has no intrapeptide hydrogen bonds, and it has previously been found to be one source of flexibility, e.g., in collagen and elastin, as well as an intermediate in silk processing. PLH(DBSA)$_{1.0}$ complexes were characterized by Fourier transform infrared spectroscopy (FTIR), circular dichroism (CD), small-angle X-ray scattering (SAXS), transmission electron microscopy (TEM), and differential scanning calorimetry (DSC). The PPII-like conformation in PLH(DBSA)$_{1.0}$ is revealed by characteristic CD and FTIR spectra, where the latter indicates absence of intrachain peptide hydrogen bonds. In addition, a glass transition was directly verified by DSC at ca. 135 °C for PLH(DBSA)$_{1.0}$ and indirectly by SAXS and TEM in comparison to pure PLH at 165 °C, thus indicating plasticization. Glass transitions have not been observed before in polypeptide-surfactant complexes. The present results show that surfactant binding can be a simple scheme to provide steric crowding to stabilize PPII conformation to tune the polypeptide properties, plasticization and flexibility.

II Self-assembled polypeptide-surfactant complexes are usually infusible solids in the absence of solvent and do not allow fluidlike liquid crystallinity even when heated, which seriously limits their polymer-like applications in the solid state due to processing problems. This work is partly inspired by nature's liquid crystalline processing of silk and subsequent structural interlocking due to β-sheet formation. We demonstrate here polypeptide–surfactant complexes that are fluidlike liquid crystalline at room temperature with hexagonal cylindrical self-assembly. The hexagonal structure with α-helical polypeptide chains is then partially converted to lamellar self-assembly with β-sheet conformation through thermal treatment. We use poly(L-lysine)-dodecylbenzenesulfonic acid complexes, PLL(DBSA)$_x$ (x = 1.0-3.0), where the branched dodecyl tails suppress the side-chain crystallization. In the stoichiometric composition, x = 1.0, there is one anionic DBSA molecule ionically complexed to each cationic lysine residue. Such a PLL(DBSA)$_{1.0}$ is an infusible solid material at all temperatures until degradation. Introduction of additional DBSA, i.e., x = 1.5 or 2.0, plasticizes the material to be shear-deformable and birefringent. In organic solution, as witnessed by small-angle neutron scattering (SANS), the PLL(DBSA)$_x$ complexes form bottle-brush-like cylinders, which upon evaporation of the solvent self-assemble into hexagonal cylindrical morphology with α-helical PLL secondary structure. Heating of PLL(DBSA)$_x$ with x = 1.0-2.0 up to the range 120-160 °C leads to the formation of lamellar self-assembled domains with β-sheet conformation of PLL, which coexist with the hexagonal self-assembled structures with α-helical conformation, as shown by Fourier transform infrared spectroscopy (FTIR) and small-angle X-ray scattering (SAXS). Higher complexation ratio, i.e., x = 3.0, results in soft and shear-deformable hexagonally packed cylinders at room temperature, but heating irreversibly converts the PLL to a random coil conformation, which leads to a disordered structure. The present model studies show that in polypeptide-surfactant self-assemblies it is possible to change the properties of the material by thermal treatment due to irreversible structural and conformational transformations.

III This work describes the solid-state conformational and structural properties of self-assembled polypeptide–surfactant complexes with double-tailed surfactants. Poly(L-lysine) was complexed with three dialkyl esters of phosphoric acid (i.e., phosphodiester surfactants), where the surfactant tail branching and length was varied to tune the supramolecular architecture in a facile way. After complexation with the branched surfactant bis(2-ethylhexyl) phosphate in an aqueous solution, the polypeptide chains adopted an α-helical conformation. These rod-like helices self-assembled into cylindrical phases with the amorphous alkyl tails pointing outward. In complexes with dioctyl phosphate and didodecyl phosphate, which have two linear *n*-octyl or *n*-dodecyl tails, respectively, the polypeptide formed antiparallel β-sheets separated by alkyl layers, resulting in well-ordered lamellar self-assemblies. By heating, it was possible to trigger a partial opening of the β-sheets and disruption of the lamellar phase. After repeated heating/cooling, all of these complexes also showed a glass transition between 37 and 50 °C. Organic solvent treatment and plasticization by overstoichiometric amount of surfactant led to structure modification in poly(Llysine)–dioctyl phosphate complexes, PLL(diC8)$_x$ (x = 1.0-3.0). Here, the α-helical PLL is surrounded by the surfactants and these bottle-brush-like chains self-assemble in a hexagonal cylindrical morphology. As x is increased, the materials are clearly plasticized and the degree of ordering is improved: The stiff α-helical backbones in a softened surfactant matrix give rise to thermotropic liquid-crystalline phases. The complexes were examined by Fourier transform infrared spectroscopy, small- and wide-angle X-ray scattering, transmission electron microscopy, differential scanning calorimetry, polarized optical microscopy, and circular dichroism.

IV We report on highly ordered oblique self-assemblies in ionic complexes of PEGylated triple-tail lipids and cationic polypeptides, as directed by side-chain crystallization, demonstrating also reversible oblique-to-hexagonal order-order transitions upon melting of the side chains. This is achieved in bulk by complexing cationic homopolypeptides, poly-L-lysine (PLys), poly-L-arginine (PArg) and poly-L-histidine (PHis), in stoichiometric amounts with anionic lipids incorporating two hydrophobic alkyl tails and one hydrophilic polyethylene glycol (PEG) tail in a star-shaped A_2B geometry. The polypeptides fold into two different helical conformations in the complexes, namely α-helix (PLys, PArg) and PPII-type helix (PHis). Aiming at periodicities at different length scales, i.e. hierarchies, the PEG tails were selected to control the separation of the polypeptide helices in one direction while the alkyl tails were selected to determine the distance between the hydrophilic polypeptide/PEG layers, resulting in an oblique arrangement of the helices. We expect that the high overall order, where the self-assembled domains are in 2D registry, is an outcome of a favorable interplay of plasticization due to the hydrophobic and hydrophilic lipid tails combined with the shape persistency of the peptide helices and the crystallization of the lipid alkyl chains. Upon heating the complexes over the melting temperature of the alkyl tails, an order-order transition from oblique to hexagonal columnar morphology is observed. This transition is reversible, i.e., the oblique structure with 2D correlation of the helices is fully returned, implying that the alkyl tail crystallization guides the structure formation. The competition between the soft and harder domains teaches on concepts towards well-defined polypeptide-based materials.

V Novel hierarchical nanostructures based on ionically self-assembled complexes of diblock copolypeptides and surfactants are presented. Rod-coil diblock copolypeptide poly(γ-benzyl-L-glutamate)-*block*-poly(L-lysine), PBLG-*b*-PLL (M_n = 25 000 and 8000 for PBLG and PLL, respectively, polydispersity index 1.08), was complexed with anionic surfactants dodecanesulfonic acid (DSA) or dodecyl benzenesulfonic acid (DBSA), denoted as PBLG-*b*-PLL(DSA)$_{1.0}$ and PBLG-*b*-PLL(DBSA)$_{1.0}$, respectively. The complexation leading to supramolecular rod-comb architectures was studied by transmission electron microscopy (TEM), small-angle X-ray scattering (SAXS), Fourier transform infrared spectroscopy (FTIR), and polarized optical microscopy (POM). PBLG-*b*-PLL, PBLG-*b*-PLL(DBSA)$_{1.0}$, and PBLG-*b*-PLL(DSA)$_{1.0}$ self-assemble with alternating PBLG lamellae and PLL-containing lamellae with a periodicity of 27-33 nm. Within the PBLG lamellae, the rod-like PBLG helices pack with a periodicity of ca. 1.3 nm. The internal structure of the PLL-containing lamellae depends on the complexation. For pure PBLG-*b*-PLL, the PLL chains adopt a random coil conformation and the PLL domains are disordered. For PBLG-*b*-PLL(DSA)$_{1.0}$, lamellar self-assembly of periodicity of 3.7 nm within the PLL(DSA)$_{1.0}$ domains is observed due to crystalline packing of the linear *n*-dodecyl tails. For PBLG-*b*-PLL(DBSA)$_{1.0}$ with branched dodecyl tails, a distinct SAXS reflection is observed, suggesting self-assembly within the PLL(DBSA)$_{1.0}$ domains with a periodicity of 2.9 nm. However, due to the absence of higher order reflections, the internal structure cannot be conclusively assigned. The efficient plasticization which leads to fluid-like liquid crystallinity in PBLG-*b*-PLL-(DBSA)$_{1.0}$ and an α-helical conformation according to FTIR allows us to suggest that the PLL(DBSA)$_{1.0}$ domains have a hexagonal internal structure. The interplay of self-assembly at different length scales combined with rod-like liquid crystallinity can open new routes to design functional materials.

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